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XPERIMENTAL INVESTIGATIONS WITH TRYPAN BLUE IN CONNECTION WITH THE INTRASPINAL TREATMENT OF LUES NERVOSA.

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EXPERIMENTAL INVESTIGATIONS WITH TRYPAN BLUE IN CONNECTION WITH THE INTRASPINAL TREATMENT OF LUES NERVOSA

ACADEMISCH PROEFSCHRIFT

TER VERKRIJGING VAN DEN GRAAD VAN DOCTOR
IN DE GENEESKUNDE AAN DE UNIVERSITEIT
VAN AMSTERDAM, OP GEZAG VAN DEN RECTOR
MAGNIFICUS PROF. S. MENDES DA COSTA, HOOGLEERAAR IN DE FACULTEIT DER GENEESKUNDE.
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Aan mijn Ouders.

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Daar gewoonlijk de promotie beschouwd wordt als het eindpunt der academische studie, voel ik 't als een noodzakelijkheid rustig te herdenken en te bedanken hen, die tijdens mijn studententijd mijn leeraren en voorlichters zijn geweest.

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INTRODUCTION.

The views on the pathogenesis of tabes and dementia paralytica have changed greatly during recent years owing to modern investigations. Whereas up till a short time ago these diseases were considered as metaluetic affections in which the changes in the nervous system were not considered as directly caused by the syphilitic virus, gradually, but certainly another view is coming to the foreground. The discovery of the spirochaeta pallida in the central nervous system of these patients, the results of the examination of the blood and the cerebrospinal liquid and the fact that syphilitic symptoms could be raised experimentally in animals by means of injections of blood or nervous tissue of these patients, increases the number of those who see in tabes and dementia paralytica genuine active syphilitic diseases of the nervous system.

Consequently it is not surprising that the hope has increased that these diseases might be influenced therapeutically. Especially since the discovery of salvarsan made the therapy of syphilis more intense, many alienists and neurologists have taken up again the investigation.

The antiluetic therapy did not only consist of a systematical experimenting of several antiluetic drugs upon men, but these efforts were also based upon pharmacological investigations. Especially have the investigations of Goldmann been a guide to many. Mott, the wellknown worker in the dominion of neurosyphilis has repeatedly drawn attention to the great importance of Goldmann's work in practical neurology. In particular the efforts to increase the action of the antiluetic drugs upon the central nervous system, bij endolumbal application, are based upon Goldmann's work. Many have effectually taken up the endolumbal therapy and many favourable results have been communicated from numerous sides.

Rather in contradistinction with these results, were the results, obtained in Holland (L. Bouman, Meyers, and others) and especially in the Wilhelmina Hospital many difficulties were to be overcome and disagreeable experiences were made. Before continuing in this way and before subjecting another series of patients with tabes and dementia paralytica to this therapy, I thought it desirable to ask, whether the present line had sufficient scientific grounds. I therefore repeated and extended Goldmann's experiments. From these I have come to the conviction that the relations are otherwise than he has given them. Further I have studied the chemical and physical characteristics of the antiluetic drug most used at the present time, viz, neosalvarsan, and in connection with further experiments made with this preparation, I have obtained different view into its properties, with regard to an eventual influence on processes in the central nervous system, than is generally given.

Succesively I will treat the following questions in this paper:

First, with the aid of literature and my own considerations, I will study which standpoint is best founded with regard to our present knowledge of the origin of the cerebrospinal fluid in connection with the function of the choroid plexus and of the different lymph-paths in the central nervous system. In the following chapter I will give a summary of Goldmann's work. I will elucidate its importance, also in connection with the questions arising in the first chapter.

In the third chapter I will study the endolumbal and endocranial method of treatment.

After this I will give a description of my own experimental investigations and elucidate in the fifth chapter the importance of these experiments in connection with the theory of vital staining.

In the sixth chapter I intend to consider the method of the endolumbal injection as a method of treatment, also in connection with the results of own investigations. In the same chapter I will discuss some observations made in the Wilhelmina Hospital.

CHAPTER I.

On the cerebrospinal fluid and the protecting membranes of the central nervous system.

The particular place which the cerebrospinal fluid holds in relation to the other fluids of the body, has urged most investigators to accept a specific origin of this liquid.

Their argumentation was especially based upon two facts.

1) The composition of the fluid, which differs widely from that of other fluids of the body.

2) The fact that some substances, which are injected in a large quantity into the blood or lymphatics, cannot be indicated in the liquid.

Ad I. The cerebrospinal liquid is a limpid fluid, containing very little protein, some anorganic salts, a small quantity of glucose and a few ferments.

Thus there is a great difference with the composition of the lymphe, e.g. the latter contains much more (0.3—5 %) protein, fats, etc. The H-Ion concentration of the liquid is about the same as that of the blood.

Ad 2. The fact that some materials are not to be indicated in the liquid which circulate in a large quantity in blood or lymphatics, is for most investigators a proof of the specific origin of the fluid. Other materials have been also studied in this respect, viz.

A. Drugs and poisons.
B. Dyes.
C. Immune materials.
D. Ferments.
E. Metabolic products.

A Drugs and Poisons.

BROMIDES. Redlich found that Bromides only penetrate into the fluid when used for a long time, and if the meninges were intact. Rotky a.o. could not confirm this: he was able to indicate Bromides only in the fluid of patients with tuberculous meningitis.

Walther, on the contrary, working with the reagent of Schiff, succeeded in indicating Bromides in the cerebropinal fluid of epileptics, even if they got only 4 gram a day.

IODIDES. In normal cases Rotky could not indicate potassium iodide; with meningitides it succeeded. (Cruchet et Lévi).

MERCURY. Still more important than potassium iodide was the indication of mercury and salvarsan in the fluid.

Von Jaksch and Rotky with cures of mercury never found a trace of it.

In chronic poisoning by mercury Sicard and others have neither been able to indicate it in the cerebrospinal fluid.

SALVARSAN. Immediately after the introduction of salvarsan this was looked for in the fluid, especially in connection with the antiluetic therapy of general paresis.

Negative results were obtained by Engmann and Camp, whilst Sicard, with the assistance of Marsh's apparatus succeeded in indicating arsenic in the fluid after intravenous application of salvarsan. Positive results were likewise obtained by Hall, Benedict, and others.

The great number of authors, who have studied those questions, shows that most of them attribute supreme importance to the presence of drugs in the fluid, as a condition of neurotherapeutic reaction.

Macintosh and Fildes do not share this opinion and I, too, doubt the importance of those facts on grounds to be mentioned later.

UROTROPIN. Urotropin passes easily into the fluid (Crowe). Weinrich even studied the hydrocephalus by means of this drug.

SALICYLATES. Heinsius van den Berg indicated antipyrine; acidum salicylicum does not seem to penetrate. Chloroform and aether may be found in the cerebrospinal fluid (Schumm, etc.)

B. Dyes.

Uranin proved to penetrate into the fluid (Kafka), methylen-blue only in uremia (Castaigne).

Macintosh and Fildes injected into rabbits intravenously all kinds of dyes, and studied both the staining of liquid and nervous tissue. After the injection of dyes which stain the nervous tissue as well as after the injection of u dyes which do not, the cerebrospinal fluid proved to remain colourless.

The absence or presence of dyes in the cerebrospinal fluid thus proved to be no essential condition for the staining of the nervous tissue. From this we may conclude that neither the presence nor absence of a drug in the fluid forms a condition for its penetration into the nervous tissue.

C. Immune materials.

Under normal conditions no hemolytic amboceptors are found in the fluid. In general paresis their presence has been used by Weil and Kafka as a diagnostic test.

As to other anti-toxins, agglutinins, etc. Kafka accepts, on the ground of the investigations of Ransom, Becht and Greer, that they may pass into the fluid, be it then in a small quantity.

Whether this penetration must be considered as a mechanic process, is still doubtful in my opinion. I think it better to consider the presence (the formation) of these specific anti-toxins rather as a local reaction on the presence of bacteria or bacterial products. (In the lymphe, too, the anti-toxins react rateh late during an infectious disease).

A definite opinion about these matters is not to be formulated as yet, since the publications are usually too fragmentary. There are, for instance, no indications about the duration and nature of the infection, the localisation of the bacteria, etc.

D. Ferments.

On the ferments of the fluid few investigations have been made, though the knowledge of them is of great importance.

In normal conditions an oxydating enzyme (oxydase) was found by Pighini, which most probably is to be identified with the cerebrospinase of Cavazzani.

Szabo found katalase and lipase in the fluid of patients with general paresis. 1).

The importance of these investigations is especially the fact that cholin, a product of lecithin, has been found

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1) He draws attention to he fact that the enzyme contents of the cerebrospinal fluid is higher in general paresis thans in normal conditions. His u paper gives copioes information on the ferments of the fluid.

in the fluid of patients with organic nervous diseases whilst Gennerich considers the penetration of liquid and ferments into the nervous parenchym as the cause of the degenerative changes in paresis. Other students also accept a connection between the enzymes of the fluid and the decomposition of the nervous system.

A diastatic ferment was found by Wohlgemuth and Sceczi. Perhaps its presence in the fluid may be of value for the metabolism of the nervous parenchym, in connection with Mott's considerations about the convertion of sugar into neural energy.

Leschke and Pincussohn demonstrated a glycolytic ferment.

On the other hand the defending ferments of Abderhalden could not be found.

E. Metabolic products.

Apart from cholin and lecithin other metabolic products have been indicated in the fluid.

ACETON. The presence of aceton in the fluid is studied by means of the reactions of Frommer and Lieben-Mauban.

In this way Bousquet, Derrien and others succeeded in indicating aceton in the fluid of diabetics. They assumed that whenever aceton could be indicated in the blood, it will also appear in the fluid.

Aceton could be found in the fluid of patients with delirium tremens and epilepsy (Eskuchen).

Experimentally aceton could not be introduced into the fluid along the blood. Koopman, after injecting subcutaneously large quantities of aceton into rabbits, was never able to indicate it in the fluid.

UREUM. The amount of ureum in the fluid was studied by Granat. In cases of severe uremia he found 0.2% of it. 1)

GLYCOSE. Under normal conditions the fluid contains less glycose than the blood. With meningitis, however, the quantity of glycose increases, whilst also diabetes elimi-

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1) Especially in young children, the study of the ureum amount of the cerebrospinal fluid has been of great importance. Nobécourt and others (Koch, Stransky) found an increases of ureum in acute diseases of young children (pneumonia, paedatrophia, etc.).

nates the difference between the fluid and blood (Hopkins).

BILE PIGMENTS. With chronic Jaundice Widal, Sicard and Ravaut found a pigment in the fluid, which they could not identify with bile-pigment.

Mosny et Javal, on the contrary, consider the pigment as a bilepigment, just as Schotmüller and Koopman do.

CHOLESTEROL. Especially Pighini studied the cholestorol contents of the cerebrospinal fluid. He found cholesterol in the fluid of patients with general paresis, schizophreny, etc. Whether a connection exists between the presence of cholesterol and the Wassermann reaction can not yet be said.[1]

Thus we have seen that some substances penetrate into the fluid after introduction via the blood, whilst other do no.

The question now arises on what the remarkable fact is founded, that the cerebrospinal fluid occupies such a special place with regard to the other liquids of the body.

This fact has given rise to the opinion that the fluid is secreted in an entirely different way, viz, by the choroid plexus.

On the Choroid plexus:

Luschka has already considered the plexus as the producer of the cerebrospinal fluid and since his publication a great many investigations are based upon this hypothesis.

Students have tried to find an exact demonstration of the origin of the fluid founded upon experimental methods.

As such they used:

1) The histological method. (Yoshimura a.o.)
2) The physiological method. (Dixon and Halliburton)
3) The pathological method. (Mott)
4) The embryological method. (Weed).

1) Histology attracted the greatest interest. Schlaepfer studied particularly the secretory changes in the plexus-cell with the aid of the supravital method: the surviving plexus was put immediately after the removal, in a solution of dyes

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[2] Lorenz considers the changes of fat metabolism which are the cause of a positive Wassermann reaction, as founded upon an increase of the amount of lipase.

(congo-red, methylen-blue, etc.) He observed in the plexus-cells little granules, globoplasts, which conflued to droplets and leave the cell as secreta. Whereas Schlaepfer tried to compute the living condition as much as possible by means of his supravital method, the pupils of Obersteiner studied more especially the fixed and stained image of the plexus-cell under normal and pathological conditions.

Yoshimura found vacuols in the epithelia of the plexus and succeeded in demonstrating the presence of glycogen, lecithin, fibrin, etc. He was also able to demonstrate the same substances in the cerebrospinal fluid.

Hworostuchin, putting the method of Altmann into practice, found as a pro-stadium of the granules, chondriomites (very small rods lying in the cytoplasm).

Analogous facts were found by Imahura, Loeper, and other authors.

The histochemical investigations of Goldmann also plead for the secretory function of the plexus.

He, like Loeper, and able to indicate glycogen in the plexus, as well as in the central nervous system itself.

In connection with the fact that glycogen occurs in the plexus-cells, he concludes that the „intranervous" glycogen originates in the plexus and, after passing the cerebrospinal fluid, is stored up in the nervous tissue. He refers to the investigations of Mott, who invariably found glucose in the fluid, and to those of Loeper, who indicated glycogen in the fullgrown plexus of diabetics, together with a large amount of sugar in the cerebrospinal fluid.

2) The physiological investigation of the function of the plexus was also done in various ways. Some students (Capeletti, Pettit et Girard) endeavoured to find changes in the plexus, after the application of poisons which generally activate secretory processes.

The addition of pilocarpine and muscarine changed the structure of the protoplasm of the plexus-cells. Analogous results were recorded by Ciaccio and Scaglioni, who observed an increase in the number and the size of the vacuols.

Dixon and Halliburton, as well as Kramer, tried another way of investigation: they ascertained how much cerebrospinal fluid flows in a certain time from a canule introduced into the arachnoïd cavity.

It appeared that an intravenous injection of extracts of the plexus itself and of the brain, was able to excite the secretory function of the plexus.

The fluid was decreased bij extracts of the hypophysis, the pineal body, the pia, etc.

The increase of the secretion is, according to Dixon, a result either of the action of certain hormons on the plexus, or it is based upon the mechanical effect of vascular or respiratory conditions.

It must be remembered, however that the plexus is innervated by the sympathetic nervous system, which is sensitive to pilocarpine, muscarine, etc. Thus we may assume that the sympathetic nerves of the plexus are excited by the extracts and drugs mentioned above.

That no influence may be ascribed to the change of the respiration is proved by the fact that also, if artificial respiration be employed, the changes in the secretion of the plexus remain. Changes in the vascular conditions also may be excluded, because drugs which lower the blood pressure do not intensify the current.

It will be well to accept in the extracts mentioned above a substance (cholesterin?), which is able to intensify the function of the plexus.

Frazier found that thyreoid extract has an inhibitory action on the plexus, whilst Moore studied those facts in an experiment furnished by nature: with a fractura baseos cranii much liquid flowed out of the ear. Adrenaline and pilocarpine proved to intensify this outflow.

3) Pathological evidence in favour of the secretory function of the plexus has been given, among others, by Mott, who describes hydrocephalus caused by occlusion of the fourth ventricle.

Also Dandy and Blackfan, who experimentally made an occlusion of the fourth ventricle, saw an acute hydrocephalus arise.

4) Concerning the ontogenetic arguments for the secretory function of the plexus, Weed found that inward secretion of the choroid cells is the only secretion up to an embryo of 14 m.M.

It was proved that in embryos 14 m.M. in length cerebrospinal fluid also passes from the ventricles to the peri-axal spaces.

It becomes incumbent to regard such an extraventricular spread of the fluid as an indication that the production of the fluid within the cerebral ventricles exceeds their capacity.

It seemed necessary to correlate the extraventricular spread of the fluid with the morphology of some intraventricular structures at the critical stage of 14 m.M. in the pig-embryo.

The first evidences of villous tufting in the choroid plexus of the fourth ventricle were found to occur at this stage in the pig. 1)

I have thus given, after a discussion of the facts which speak for the special place of the cerebrospinal fluid, a brief summary of the arguments delivered for the secretory function of the plexus in the formation of the fluid.

As the secretory function of the plexus is denied by many authors, I shall give a short review of the other theories, which have been given instead of the plexus theory.

Spina considers the fluid as a specific product of the brain. 2)

To prove this he made a prolapse of the brain, injecting adrenal extract after section of the oblongata, and observed that a clear fluid appeared.

Even intoxication with atropine could not prevent the formation of droplets on the surface of the brain, which, according to Spina is a serious argument against the secretory function of the plexus. This view is confirmed bij Lewandowsky whose opinion is based upon the fact that the fluid contains more potassium salts than the blood, whilst the brain also contains a large quantity of potassium.

Salkowsky has refuted these arguments by indicating that the amount of potassium in the fluid changes very much. Also Myers could note little variation in the potassium estimations during life.

Even with degenerative processes of the nervous system the potassium of the fluid is not necessarily increased (Rosenbloom).

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1) The ontegenetic process described by Weed is in harmony with phylogenetic phenomena.

Kingbury, Studnicka and Kappers, have called attention to the fact that in lower vertebrates the inversion of the plexuses seldom occurs. A very striking protrusion f.i. is found in ganoids.

Kappers connected this with the absence of liquor cerebrospinalis externus in fishes, the function of the plexus thus being confined to inward secretion, that evidently can attain such a degree that the plexus membranes protrude outward over the brain wall, along the whole brain stem.

2) In connection with this theory it may be mentioned that Nageotte, Mawas and Achucarro have called attention to a secretory function of the glia cells.

I may add to this that the relation between K and Na in the fluid and in the brain are very different. (more Na than K.)

Another view, viz. that the fluid might be a common transudate, has been defended by Wohlgemuth and Sceczi.

They were able to prove that after ligature of the pancreatic ducts, the quantity of diastatic ferment in experimental oedema runs parallel with that of the cerebrospinal fluid, whilst AUREL and BABES, by comparing the cerebrospinal fluid with cedematic fluid (in cases of decompensatio cordis) found agreeing figures.

Rather strange is Head's conception: „We believe that the subarachnoid space is a closed cavity.... It is filled with a relatively stagnant cerebrospinal fluid which acts simply as a water cushion to the spinal cord and brain stem." (p. 19)

He considers the fluid as a product of the epithelium, lining the arachnoid spaces, and doubts whether the ventricular fluid contributes to the composition of the cerebrospinal liquid.

A proof against the mere transudation theory was furnished by Sturz-Berg, who indicated that the secretion of the fluid did not increase after ligature of the neck.

The question of the separation between ventricular fluid and cerebrospinal fluid as two different liquids, has been treated by Weed and Cushing.

According to Weed a part of the external cerebrospinal liquid proceeds from transudation from the vessels into the perivascular system. On the other hand, Grynfelt et Euzière ascribe a secretory function in the formation of the internal fluid to the ependymal cells. They studied the changes which take place in the cells of plexus and ependyma during the secretion of liquor cerebrospinalis, and observed in both cells analogous phenomena.

Leblanc studied the ependyma of reptiles, and found that when the animals were kept for I or 2 × 24 hours in a refrigerator, it proved that the chondriomes had disappeared from the ependyma.

Kappers found distinct secretory places in the ependyma of the tweenbrain in several vertebrates, especially reptiles.

Other functions of the plexus.

Pellizi ascribes to the plexus a resorbing function. He finds in the plexus during a long period of foetal life, numerous granulated cells filled up with fat droplets. (tripalmitin)

The same droplets seem to appear in the epithelia of the plexus. He considers the granulated cells as „Abraumzellen" which have taken up katabolic products, resorbed from the cerebrospinal fluid by the plexus epithelia.

After birth all kinds of substances appear in the plexus epithelium and in the connective tissue of the plexus, such as fats, lipoids, pigments, etc.

Probably these substances arise in consequence of the function of the nervous tissue, and are resorbed from the cerebrospinal fluid by the plexus. If the metabolism of the nervous system was excited, e.g. by acute intoxication or operations which exposed the convexity of the cortex, the quantity of the above-mentioned products was increased in a great degree.

Probably the plexus has the function to take away the katabolic products from brain-activity. If the plexus was extirpated (in frogs), rigidity occurred, perhaps as a consequence of auto-intoxication.

Dustin after making experimental lesions of the nervous-system, saw a great increase of lipoid droplets in the plexus cells, which after some time proved to pass over into the axis of the villi. A similar process takes place after injection of fats into the arachnoidal spaces.

The metabolic products enter the fluid, are taken up by the plexus and brought again into circulation.

The results of Klestadt are also in favour of the resorptive function of the plexus. Carmine, glycogen, etc. injected into the ventricles appear to be resorbed by the plexus-cells.

He supposes that also the glia-cells resorb actively the fat-substances from the blood and give them to the nerve cells.

If we summarize the facts mentioned above, it proves to be necessary to accept a specific origin of the cerebrospinal fluid.

Most investigators accept the plexus as originator of this fluid, especially on the ground of histological studies.

Some among them consider the fluid as a specific secretion of the brain, whilst Weed accepts a double origin, one for the internal and one for the external.

On the glia membranes and the lymph-spaces of the N.S.

Since in the cerebrospinal fluid many substances cannot be indicated, which may be demonstrated in the general circulation, most investigators think it necessary to accept the presence of a physiological protecting membrane for the central nervous system.

Goldmann (see chapter II) considers the choroid plexus as such.

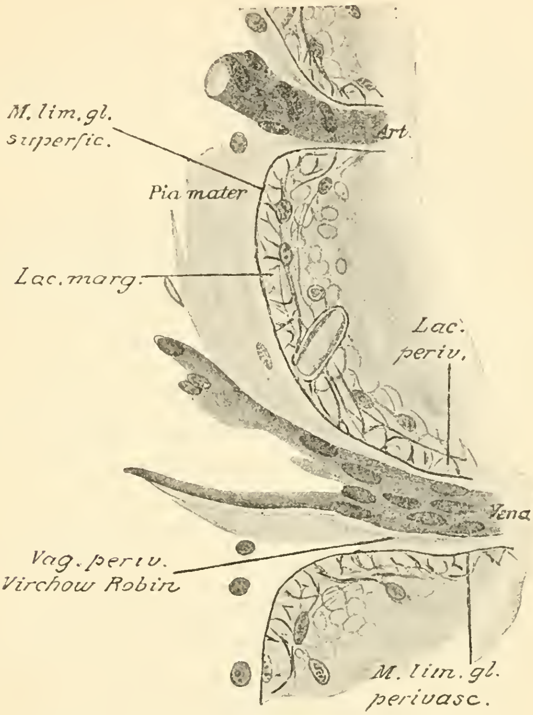
The fact that besides the vessels which occur in the choroid plexus, so many others penetrate into the central nervous system, made other workers look for other membranes which have the function of protecting the nervous tissue against perilous materials.

The investigations of Held especially have been of great value to this question. He examined chiefly the distribution of the glia, the glial membranes and the spaces of the nervous tissue.

His had already found in the embryo, that by the flattened offshoots of the spongioblasts a myelospongium is formed, which bounds like a „Randschleier" the cerebral tube.

Held found this „Randschleier" to appear in adults also as the membrana limitans gliosa superficialis.

Fig. 1.



Glia membranes in the cortex cerebri (HELD).

It consists equally of the flattened offshoots of glia-cells which surround the central nervous system by an uninterrupted membrane. This membrane follows the vessels which penetrate into the nervous system, and is even found around the finest capillaries. (fig. 1)

In this way a separate sheath is formed round the vessels, the membrana limitans gliae perivascularis, which forms a complete boundary between mesenchym and nervous parenchym.

That this membrane forms a complete partition may be proved from injections made by Held. After intraparenchymatous injections of chinese ink the black particles proved not to pass the glia membranes.

As these membranes consists of the offshoots of glia cells, we may ascribe to them, besides a protective, also a metabolic function.

Regarding the lymph spaces in the central nervous system itself, I can state, that Robin and Virchow were the first authors who described perivascular spaces.

Held gives the following description of these: the most central part of the pia is the intima piae, a membrane consisting of „Haütchenartige Grenzzellen". This membrane follows strictly the membrana limitans gliae, with which it is nearly glued together.

The arachnoid cavity now continues immediately into Virchow-Robin's space, which is bounded at one side by the adventitia of the vessels if this occurs and at the other side by the complex intima piae + membrana limitans gliae perivascularis.

I must draw attention to the fact (in view of the prevailing confusion in the nomenclature) that the intima piae, as soon as it passes into the nervous tissue itself, is called the membrana limitans accessoria.

As to the vessels of the white substance I may state that the adventitial space increases, so that between adventitia and membrana limitans accessoria a space is formed, the so-called „Zirkumadventitielle Raüme."

These are only extensions of Virchow-Robin's space.

Probably those spaces are continued round the vessels of the whole nervous system, even round the capillaries, to attain finally round the veins another extension. In this way Held described a liquid system which runs through the whole nervous tissue.

He, however, in his publication, does not speak of any direct contact with the so-called pericellular spaces.

This question, which is an important one viz. the presence of pericellular spaces and their connection with the spaces of Virchow-Robin, is now shortly to be treated.

Obersteiner is absolutely convinced of the existence of such spaces round the cells, on the ground of injections.

Mott studied the question in another way: he caused anaemia cerebri with monkeys bij ligature of nearly all the arteriae of the brain. The so-called pericellular, or perineuronal, spaces proved to be distinctly visible.

Those spaces were studied by many authors in cases of sarcoma and carcinoma of meninges and brain (Rach, Merzbacher). Round some pyramidal cells they found tumor elements, which fact is interpreted by accepting the presence of pericellular spaces.

Weed made injections of ferrocyannatrium into the brain substance and observed dye-granules in the spaces mentioned above.

Criticism was not lacking: Held ascribed the formation of these spaces to the use of certain fixation solutions, by which shrinkage of the surrounding tissue is caused. By making use of other methods of fixation their formation may be prevented. (Bruce and Dawson).

Kooy, by making use of celloidin, in a case of sarcoma piae matris, succeeded in avoiding the formation of the pericellular spaces, and concludes from his investigations that no communication exists between the perivascular and so-called pericellular spaces, and does not accept the existence of the latter.

Consequently Mott's supposition that the ganglion cells are in direct connection with the cerebrospinal fluid is to be discarded.

But how then is the metabolism of the ganglion cell regulated?

The glia cells with their offshoots penetrate into the nervous tissue and surround the ganglion-cells.

It has been proved by experiments of Wlassak and Krückmann that myeline and fat substances, in pathological conditions, are transported via such glia cells. The alimentation of the ganglion cells may take place in an analogous way. (c.f. also Behr).

Probably the trophospongium of Holmgren has something to do with those processes.

The membrana limitans gliae superficalis may, therefore probably be considered as an active „Uebertragungsapparat" of aliments from the adventitial spaces to the nervous cells, and at the same time as a protective organ against the penetration of poisons into the nervous system.

Resuming, we may conclude that in the central nervous system a canal system exists, which is in direct communication with the arachnoid space. This canal system makes an intensive circulation of fluids possible, and is separated from the nervous tissue itself by a gliatic membrane.

Only some materials may penetrate by diffusion into the nervous parenchym. Whether colloid materials may be able to penetrate into the parenchym will be treated afterwards.

This conception is supported by the experiments of Flexner and Amoss on „aseptic meningitis."

They caused an aseptis meningitis by intraspinal injection into monkeys of serum, or normal saline.

If polyomyelitic virus was applied intravenously without causing this meningitis, no polyomyelitis could be seen. It succeeded however if a meningitis had preceded. This may be interpreted by accepting that the glia membranes have lost their protective function by the injection of serum, etc.

In 1910 Sicard and Salin drew attention to this form of meningitis, whilst Mestrezat and Sappey obtained o felectromercurol an increase of the menigeal permeability by injection.

Lewandowsky believes that we have to consider the vessel wall as the impermeable membrane of the nervous system. This opinion, however, was again abandoned by him in 1916.

That a drug can act much more intensely after intraspinal injection than after intravenous application, may be explained in another way: After intraspinal injection a certain quantity of poison is brought into direct contact with the nervous system. In order to send the same quantity to the nervous system via the circulation, the dose must be much larger, since large quantities are stored by the other organs.

And if after intravenous application a small quantity of poison is sufficient to raise symptoms from the side of the nervous system, this must be based upon a particulair affinity of this poison to the nervous system.

According to Lewandowsky the absence of drugs in the cerebrospinal fluid is a direct result of the principle of distribution of the drugs.

So far I have endeavoured to give a survey of what is known about the penetration of drugs, etc., from the circulation into the cerebrospinal fluid.

After having drawn attention to the special character of the fluid, I have tried to explain why many materials which are introduced into the blood, cannot be indicated in the fluid.

In this connection I have described the function of the choroid plexus and of the glia membranes.

Conclusions from the facts mentioned in the preceding pages will be given in the critical part of this paper.

In the following pages I shall communicate shortly what is known about the currents in the liquor cerebrospinalis.

Pathway of the Fluid.

The first who studied those currents of the cerebrospinal fluid was Quincke.

He injected intraspinally (in dogs) a cinnaber emulsion, and observed in 10 or 12 cases that in a short time the dye had penetrated into the cranial cavity and had stained the base of the brain.

The dye could also be seen along all the cranial and spinal nerves. After intracranial injection of the dye, the greater part of it was found also at the base.

The dye granules were generally stored in large lymphocytes. In the nervous tissue cinnaber was never to be found.

Quincke concluded from those facts that there is a connection between the arachnoid spaces of brain and spinal cord.

He considered the gathering at the base partly as a consequence of gravitation, partly of the direction of the currents in the fluid, which is from the spinal cord to the basis of the brain.

Key and Retzius obtained analogous results, as did Jacob and Sicard.

The latter injected emulsions of chinese ink and says:
„ces granulations noiratres.... (gagnent) assez rapidement.... la région atlanto-occipitale, le bulbe, le base du cerveau entre les pédoncules et le chiasma, plus tard les ventricules latéraux. La corticalité cérébrale n'est impregnée de noir que plus longtemps après...."

Of great importance is the fact, that he could cause typical meningitis tuberculosa of the base by injecting bacilli intraspinally.

From those facts he concludes:

„Le liquide céphalo-rachidien est dans les méningites infectieuses le facteur essentiel de la dissémination des microbes et de leurs toxines" (pag. 145).

Lewandowsky supposes on the contrary that the liquor cerebrospinalis is a stagnant fluid. The spread of injected materials, according to him, takes place by diffusion.

A current from the ventricles to the arachnoid space is accepted by Kafka. Ahrens publishes an analogous view.

Most systematically has the pathway of the cerebrospinal fluid been studied by Weed, by injecting ferrocyanide solutions.

In this series of experiments a solution of potassium ferrocyanide and iron ammonium citrate was injected endolumbally.

After completion of the injection, the brain with its meninges was fixed in a formalin solution containing one percent of hydrochloric acid. The communication between the arachnoid spaces of the brain and spinal cord could be demonstrated, whilst he was able to state the return of the fluid to the general circulation via the „arachnoidal villi."

„These villi are projections of the arachnoidea through the dural wall, prolonged directly beneath the vascular endotheluim of the venous sinuses." The process of escape of the cerebrospinal fluid from the arachnoid villus into the great sinus appeared to be a simple one of filtration or of diffusion. This might render the fact plausible that, after intraspinal application of methylen blue, the dye is to be seen in the urine after 20 — 30 minutes. (Lewandowsky).

In all cases of intraspinal injection, it had been proved that the greater part of the dye was stored at the basis of the brain.

Especially Campbell considers gravitation as one of the reasons of the fact why, after intraspinal injection, no dyes or drugs can reach the convexity of the brain (in paresis).

He therefore wants to intensify the flow from the spinal sac into the cranial cavity by raising the foot of the bed and allowing the patient's head to be at a lower level than the sacrum.

Still better is it to inject the drug into the cornu anterius of the lateral ventricle of the brain. He believes that an important part of the injected drug may reach the „upper cranial chamber", from whence it has a free path over the convexity of the brain.

A part of the solution, however, escapes downwards, leaves via the foramina Magendie et Luschka the fourth ventricle, and flows further downward through the foraminum magnum. He tried to avoid this by raising the foot of the bed.

As I thought myself that Goldmann's results (staining of the basis of the brain) could be explained by gravitation. I endeavoured to solve this problem experimentally.

Those investigations will be discussed elaborately in a following chapter. I will, however, mention the following case in order to demonstrate that after intraspinal injection in men, the dyes are stored in an analogous way as in animals.

DINA V., born 18-2-'17, entered the Wilhelmina hospital on the 10th of February.

The 9th of February she suddenly fell ill, complaining of headache. She had the typical symptoms of a meningitis cerebrospinalis. The temperature was 39.1° rectally.

11-2-'20: Urotropin — lumbar puncture: 30 c.M. of fluid removed.

The fluid was purulent. Methylen blue was injected intraspinally.

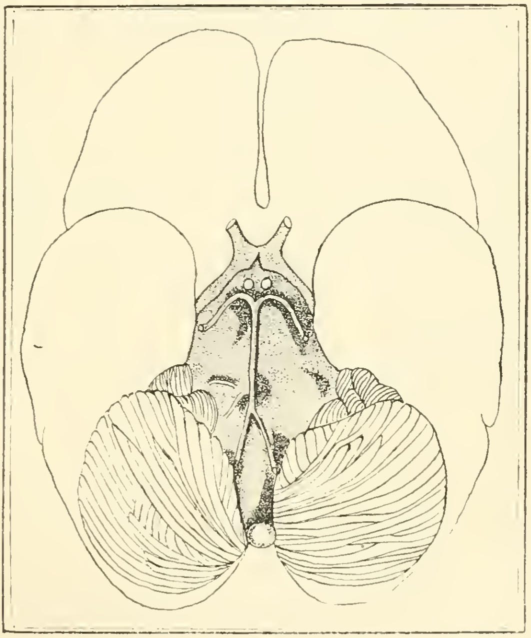
12-2-'20: lumbar puncture the fluid was still purulent, the pressure increased. Twenty c.M. were removed. Its colour was blue. Again methylenblue was injected.

13-2-'20: lumbar puncture: 30 c.M. of purulent fluid removed. Death of the patient.

Autopsy: The meninges of the spinal cord were stained intensely. A transverse section of the cord appeared to be colourless.

The base of the brain was stained at the following parts: ventral part of the oblongata, pons, pedunculi cerebri, corpora mamillaria, infundibulum, chiasma, medial part of the cerebellum (see figure II).

Fig. 2.



Base of the brain of DINA V. Intraspinal injection of methylene blue. The dotted parts are blue.

The convexity of the brain was quite colourless.

The bladder, pelvis renis and kidneys were blue, whilst also the walls of the cerebral ventricles showed a light staining.

On the penetration of drugs into the

nervous tissue itself, after intravenous application, etc.

After the discussion on the choroid plexus, the liquor cerebrospinalis and the fluid spaces of the nervous system, and in connection with the subject of this dissertation, a few short remarks may be made on the penetration of chemical materials into the nervous system itself.

Many facts on this object are known, and narcotics especially have been studied. Buchheim has already given as his opinion that narcosis was a consequence of a reaction between the nervous elements and the narcotics.

Thus it might be expected that chloroform and ether appear in a higher dosis in the nervous system than in the other organs.

Bromides (Grünwald) and antipyrine (Heinsius van den Bergh) could be indicated in the nervous tissue after continued application.

Nickel and cobalt were shown in the nervous tissue after intravenous injection (Winkler-Junius).

The same may be said with regard to strychnine, morphium, potassium iodide., etc.

Also salvarsan has been studied in this respect.

Some authors even considered the intravenous application of salvarsan as having a bad influence on the nervous tissue.

v. Marschalko and Veszpremi observed in rabbits after intravenous injections of salvarsan, hemorrhages in pons and medulla oblongata.

Doinikow and Igersheimer, however, obtained negative results, and concluded that salvarsan has no neurotropy.

Mac intosh studied this question by making use of a different method: He analysed the brain of a child, which had died after the intravenous application of salvarsan. No arsenic could be indicated, in contradistinction with the other organs, which contained arsenic to a high amount. Consequently he refuted the neurotropic action of salvarsan.

Afterwards Mac intosh and Fildes came to another conclusion:

It appeared that absence of arsenic from the brain might be due to two distinct factors. Firstly, the arsenic might not be „fixed" by the brain as already suggested, secondly, it might not gain access to the brain, owing to some peculiarity of the cerebral vessels.

„In order to test this possibility we conducted experiences in vitro, applying neosalvarsan directly to fresh brain substance, and then found that neosalvarsan was in fact „fixed" by the brain."

100 Gr. of fresh human brain and 100 Gr. of human liver were minced and placed in two separate glass bottles of 1000 c.c. capacity. These bottles were filled with saline solution, shaken, allowed to stand, and the supernatant fluid removed.

The tissue was thus freed from excess of blood; 500 c.c. of saline solution, containing 150 mGr. of neosalvarsan, were then added to each, and the bottles were shaken for an hour.

At the end of this time the supernatant fluid was removed and the bottles filled with saline solution eight times, removing the washings by decantation. By this method it was hoped that all „unfixed" neosalvarsan would be removed from the tissue. Estimations of the amount of neosalvarsan in the washed tissue, etc. were then made.

It appeared that dead brain substance had fixed considerable quantities of neosalvarsan in vitro.

It may be objected to these experiments that the arsenic was merely entangled physically with the minced tissue and was not chemically fixed. The experiment was therefore repeated, replacing the animal tissues with finest vegetable charcoal.

Although a certain degree of absorption of the neosalvarsan was observed in this case, it was not so great as with liver and brain tissue.

The following objections may be made.

Macintosh and Fildes examined the adsorption of salvarsan to dead tissues. They find that brain tissue is able to fix as much salvarsan as liver tissue, and conclude from this that the living organs (brain and liver) fix equal quantities of salvarsan.

Those conclusions are not admitted.

Because 1°) They neglect the possibility that fixation of materials to an organ is a vital process.

2°) They consider the fixation to a dead tissue as a standard for the fixation by an organ.

Concerning the test with charcoal it has been proved by Marc that the absorption depends greatly on the character of the adsorbens.

So it is probable that the colloid material (dead brain tissue) will adsorb more salvarsan than the charcoal, a hypothesis which is supported by the fact that brain and liver tissue take up nearly the same quantities of salvarsan.

The exclusion of the vital process is of the utmost importance (see below).

Macintosh and Fildes also studied the conditions which are necessary to cause a material penetrate into the nervous system.

So they observed that methylen blue, neutral red, malachite green, after intravenous injection, stain the brain diffusely; liquor and meninges were not stained.

The brain was not stained by fluoresceine, trypan red, ,,licht grün", etc.

Meninges and choroid plexus, however, after the injection of those dyes showed a definite staining.

They attempted to explain this (analogy with Overton) by the solubility of the dyes used in chloroform.

The salvarsan group is not soluble, whereas combinations of arsenic with certain anilin dyes showed a distinct solubility in chloroform. From this they concluded:

,,The present day arsenical remedies are, to some extent, inefficient in the treatment of neurosyphilis, because they do not possess the necessary solubility to allow them to pass from the blood-vessels into the brain substance.

Their relative inefficiency has nothing to do with their absence or presence from the cerebrospinal fluid."

Of the facts mentioned in the above chapter I will make use in my discussions on the intraspinal treatment.

CHAPTER II.

The experiments of Goldmann.

In this chapter, after a short introduction on trypan blue and its use as a vital dye, I shall give a description of the effects of this dye on the central nervous system as described by Goldmann. Ehrlich and Shiga on the grounds of earlier investigations by Laveran and Mesnil, wrote in 1914 on the therapeutic effect of benzidin-dyes on trypanosomiasis. Now it appeared that some of the dyes used, especially the trypan red, stained vitally the tissues of injected animals.

Nicolle and Mesnil then submitted the series of the benzidin-dyes to a comparative chemical examination in order to find a more efficacious remedy. They found among others trypan blue and trypan violet, dyes which, besides their trypanocid effects, possess in a high degree the property of staining vitally. Franke had already studied trypan red as a vital dye; he found that this dye was taken up by all organs, except by the central nervous system.

Bouffard was the first who, incited there to by Mesnil in 1906, studied the reaction of tissue-cells to trypan blue. He experimented upon mice which were stained vitally by subcutaneous injection of the dye, each animal receiving a dose of 10 m.Gr. Within some minutes after the injection Bouffard saw a distinct staining appear in the animal. First a lightblue tint was seen at the ears, then at the tail, whilst especially the visible mucous membranes became distinctly blue (particularly the conjunctiva, the mucosa iris, etc.) The urine was violet, whilst the blood-serum showed a clear blue colour. About 8-10 days after the injection the skin and mucous membranes lost their colour. The urine resumed its normal tint again in about 20 days.

At the autopsy it appeared that the kidneys and the liver were distinctly blue. The muscles, the myocard, the nervous system, the pancreas and the genitalia were colourless. Microscopical investigations by means of frozen sections yielded the following results: it proved that a part of the dye was taken up by nearly all organs in an identical way. The liver had many dye-granules in the cells of Kupffer, the spleen contained blue granulated polymorphonuclears, whilst in the lungs and digestive tract the dye was takan up by the interstitial cells.

The central nervous system was colourless.

According to Bouffard the blue colour of the organs was not only founded on the presence of cells filled with blue granules, but also the staining of the blood-serum contributed to it. The degree of the blue colour therefore depended especially on the transparency of the organs; and as the central nervous system is rich in opaque fat materials it would not look bluish notwithstanding the presence of trypan-blue in the blood-serum. The cerebrospinal fluid was always colourless, also after the injection of a high dose of trypan blue in the vascular system From this fact Bouffard concluded that in modern „chromotherapy" it is advisable to put into practice the intraspinal treatment by injecting immediately into the cerebrospinal fluid itself.

Bouffard's investigations were fairly complete, since he had also studied the nervous system, thus forming a foundation for the experiments of Edwin Goldmann.

In 1909 Goldmann published his results in Brunn's Beiträge. He made use of pyrrol — and trypan blue, and by means of the vital staining effected by these dyes studied particularly the physiological histology of the normal animal.

Up till now histology had been the study of a final condition; the way in which this condition was attained could not be studied. By means of the vital dyes it became possible to study the evolution of the histological processes also.

Goldmann realised directly the close relation between vital staining and chemotherapy, which had already been demonstrated bij Ehrlich.

The intravenous injections of pyrrolblue caused the death of the rabbits (by embolus). Trypan blue, however, proved excellently suited for intravenous application.

The organs were fixed in a solution of 10 % formol, embedded in paraffin and cut into serial sections.

Goldmann's scheme was rather simple; in the first place he would study the distribution of dyes in the normal body in various physiological conditions. So he studied the histology of the placenta and oval membrane. In his second publication Goldmann made a special study of pathological conditions, e.g. the results of intraperitoneal inoculations of bovine and other forms of tuberculosis, the experimental degeneration of the liver, and finally the development of malignant outgrowths.

His physiological results were, with respect to the intravenous injection, the same as Bouffard's. Again a clear blue-staining of the skin (subcutis) and mucous membranes was found. In the abdomen the kidneys were conspicuous by their intense blue colour, whilst in the second place the liver drew the attention by its fine blue tint. The spleen too was light blue, as was also the intestinal tract. The gravid uterus was intensely blue, where as the lungs showed only a slight staining. The heart was generally distinctly stained.

The central nervous system again remained quite white: the choroid plexus contrasted clearly, as a blue-stained membrane against the colourless nervous tissue.

In the blood no stained elements were found.

The microscopical investigation (on frozen sections) also taught us much the same as what Bouffard had found. The liver showed a distinct staining of the cells of Kupffer, while in the capsule fine blue granulated Pyrrol-cells occurred.

The liver-cells themselves were colourless, whilst the bile (after injection of a large quantity of dye) was pale blue. In the kidney especially the cells of the contorted tubuli had absorbed much dye in the form of granules, which were arranged in elongated rods.

In the intestinal tract the colour proved to be confined almost exclusively to a large quantity of Pyrrolcells. The same holds good for the pancreas, the salivary glands, the mammary, the thyroid gland, the larynx, the trachea, the esophagus.

In the testicle only the interstitial cells were intensely stained, whilst in the ovary the follicle cells showed a tint.

In the skin a blue granulation of the cells of the connective tissue was to be seen, besides the Pyrrholcells mentioned before.

The blood-vessels were usually colourless, but for an occasional faint vital staining of the membrana elastica interna.

The central nervous system after an exact microscopical examination, also proved to be free of trypan blue. The cerebrospinal liquid was now and then light blue, which could best be demonstrated by absorbing some fluid in a piece of blotting paper. The moist part then became very slightly coloured.

As is proved from the above descriptions, in almost all organs (lymphnodules, spleen, liver, connective tissue, etc.) and especially in the peritoneum, a certain kind of cells is found, which Goldmann indicated by the collective name of Pyrrolcells. This cell in point of size ranks between the large and the small lymphocytes. Its form is round and it has a large irregularly shaped nucleus which is well supplied with chromatin. The characteristic of this cell is the finely granulated protoplasm. Directly round the nucleus the protoplasm is free of granules, in the remaining part round granules of a deep blue colour appear.

Not always, however, is the cell so much filled with granules; sometimes the protoplasm seems to be quite free of them. Nor is the shape of the cell always a round one. In the cutis, the tendons and the intermuscular connective tissue, its form is generally fusiform. The remarkable quality of these cells is that they accumulate on the spots where intense metabolic processes, of a physiological or pathological nature, take place. Thus they are numerous in the neighbourhood of the muscle fibres, between the fibres of the myocard, etc. They strongly agree with the cells of Kupffer, the interstitial cells of the testicle and the reticulum cells of the lymph nodules. Goldmann considers these cells (on account of the special investigations of Maximow) as so-called "Wanderzellen". They may be identified with the klasmatocytes of

Ranvier, the "cellules rhagiocrines" of Renaut and the adventitial cells of Marchand. For a more extensive description of these Pyrrolcells and their relations to the other elements of the connective tissue the publications of Goldmann and others furnish sufficient information.

As for the investigations of Goldmann on vital staining in pathological circumstances it will be sufficient to notice that again the Pyrrolcells have strongly phagocytic and protective functions. He considers them as metabolic cells (e.g. on account of their presence in the gravid uterus).

From the foregoing we are able to realise what happens in the body when a so-called vital dy is applied.

From Bouffard's investigations as well as from those of Goldmann, it has been seen that no dye appears in the central nervous system. How much Goldmann was struck by this, is proved by the fact that, in 1913, the well-known monograph from his pen was published, entitled „Vitalfärbung am Zentralnervensystem", in which this excellent expert upon vital staining-methods, endeavoured to account for this peculiarity in an experimental way.

Like many other investigators Goldmann opines that the choroid plexus has a secretory function. The question arose whether by means of vital dyes it would be possible to get an insight into the character of this function.

For this purpose rabbits were injected intravenously, at intervals of 4—6 days, with large doses of trypan-blue.

Before the central nervous system was removed a formol-injection was made into the heart of the etherized animal. The nervous system was fixed in a 10% solution of formol and studied in frozen sections. Only the choroid plexus proved to be stained: in the first place the plexus cells themselves were filled with the fine granules, whilst also the cells belonging to the connective tissue of the plexus showed a fine vital staining.

Toxic symptoms from the side of the nervous system did not appear even after injections with large doses.

Also the hypophysis was vitally stained. In the cerebrospinal fluid, Goldmann, in contradistinction to his earlier publications, could never find trypan blue. He tries to account for this by assuming that the choroid plexus is a barrier against the entry into the cerebrospinal fluid of substances which are present in the blood, a hypothesis which is made plausible by comparing plexus and placenta.

It had been proved before that the placenta cells, which regulate the supply of glycogen for the fetus, have the property of taking up vital dyes and fixing them in such way, that they cannot penetrate into the liquor amnii. Also the plexus cell takes up glycogen and secretes it to the ventricles, from which it is distributed via the cerebrospinal fluid over the whole central nervous system, whilst also the plexus cell takes up the vital dye.

On account of these analogies Goldmann considers the plexus as a physiological protecting membrane.

„Nicht besondere Gefäszeinrichtungen oder Gefäszalterationen der Meningen sind es, die in der Norm den Uebertritt von blutgelösten Substanzen verhüten oder begünstigen, sondern die jeweilige physiologische oder pathologische Verfassung der Plexuszellen" (p. 29).

Goldmann also quotes the studies of Schmorl, who, in cases of important alterations of the choroid plexus, was able to demonstrate, both in the liquor cerebrospinalis externa and in the ventricular fluid, materials dissolved in the blood-serum. In normal circumstances they could not be indicated in the ventricular fluid, but only in the liquor cerebrospinalis externa. Dyes brought into the vascular system could not reach the nervous system on account of the specific functions of the plexus as a protecting membrane.

It was now necessary to study how the dyes reacted histologically and especially physiologically after being injected into the cerebrospinal fluid. As has been proved in chapter I analogical investigations had been made repeatedly with the same scheme.

The pharmacodynamical part of this scheme was generally as follows:

1o. What is the pharmacodynamical reaction towards substances that are injected intraspinally and whereby is this reaction to be distinguished from that after application via the blood?

2o. On which cells of the central nervous system do these substances act?

Goldmann was the first who followed a rational way of answering these questions. He made use of a substance which, beside its therapeutic qualities, has also the quality of being a vital dye. He experimented principally upon rabbits, on which the injection was made either in the upper lumbar region of the spinal cord or into the cranial subdural space.

For the endocranial injections a small trephine opening was made into the os parietale, without lesion of the dura. Subdurally a needle was inserted and the injection very prudently made.

The intraspinal injections gave more trouble; at first the spinal cord was laid bare, but soon this method, as being too radical, was abandoned for the usual lumbar puncture which, especially with young animals (with still soft ligaments), was more easy.

The animals were etherized and the puncture was made in the lumbar part of the sharply bent spinal column. The penetration into the arachnoid space was at once observed by a quick shock which went instantly in the hind-legs of the animal.

The injected quantity was very small with rabbits: a part of 1 cM^3 of a $^1/_2$ — 1% solution.

What happened after the endolumbal injection of trypan blue?

For the first moment the animal maintained its prostrate position, with apparently paralized hindlegs. Then clonic convulsions commenced in the hindlegs, which quickly spread via the trunk to the fore legs.

Often the animal started with a scream out of the narcosis, tried to rise and to move very quickly. Suddenly violent spasms appeared in the neck, which caused the animal to fall down. A moment's rest, suddenly interrupted by new and violent rolling movements in the extremities and spasms in the muscles of the face and jaws. Then again a rest of some seconds, followed by more violent spasms and movements of flight.

These symptoms lasted from twenty to thirty minutes and then gave place to a very deep coma. Death generally followed after some hours.

The same complex of symptoms was observed after endocranial application of the poison.

Thus the endo-arachnoidal injection of about $^1/_2$ c.c. of the dye solution gave rise to very serious symptoms of irritation from the side of the central nervous system. Intravenously Goldmann could inject 100 c.c. of the solution without observing any symptoms.

With the dog the results of the subdural injection were not so plain. If, for instance, 1 $c.M^3$. of the solution was injected, a slight spasm of the face-muscles was noticeable, which soon disappeared. The animal recovered completely.

If, however, 2—2$^1/_2$ c.M^3 was injected, the animal fell into a deep coma and died 17—18 hours afterwards.

Thus far the pharmacodynamical results.

As to the histological results, it appeared that the animals which were killed 12—24 hours after the injection were best suited for microscopical investigation. It proved that the spinal cord was stained darkblue over its whole length, as also at the base of the brain. The convexity of the brain was quite colourless.

The staining also reached the region of the optic nerves as far as the sclero-corneal boundary, whilst also the bulbus olfactorius and a large part of the mucosa olfactoria were coloured.

After injection from the cranium the convexity was also stained, as well as the whole spinal cord and all the cavities of the nervous system.

The microscopical investigation showed the following characteristics: the leptomeninx had obtained a diffuse blue colour, as also its septa. Besides the staining of the connective tissue there was also a vital staining of the perifibrillar glia

According to Goldmann the most striking fact was the intensely diffuse staining of the ganglion-cells.

The nucleus was always distinctly blue, whilst the finer structure of the protoplasma could no longer be recognised.

Brain-sections showed again the staining of the leptomeninx. Especially the large pyramid cells, all with their offshoots, were intensely blue.

With regard to the dog the histological results differed. Staining of the ganglion-cells could not be shown.

Goldmann ascribes these differences to the fact that with the dog larger doses react so quickly that the diffuse and extensive staining cannot take place. If he injected a rabbit endolumbally with a large dose, (1—1$^1/_2$ c.c.^{3}) this animal too died quickly with the usual symptoms of spasm, etc.

The staining of the ganglion-cells of the spinal-cord then proved to be also very confined.

If so small a dose was used endolumbally on a dog that no clinical signs were apparent, it proved, when the spinal cord was examined some days later, that the ganglion cells displayed particular alterations.

They were filled up with small blue granules, whilst the nucleus was not stained.

The results which Goldmann obtained with dogs injected endocranially with small doses of trypan blue were equally interesting.

Clinically he could indicate only mild spasmodic contractions which proved to have a transient character.

The pia again proved to be clearly blue, just as the cells which surround the cortical vessels, which exhibited a distinctly vital staining, the nucleus being colourless.

The ganglion-cells were not stained at all.

With the facts communicated above, Goldmann now tried to answer the second point of the scheme.

He arrived at the following conclusions:

The vital dyes come via the perivascular, pericapillar and perineuronal lymphpaths, as described by Mott and others, to the ganglion-cells, penetrate into them and give rise in the first place to symptoms of irritation which document themselves by the spasms, etc. described above.

Very soon this state of irritation is followed by a condition of paralysis, in consequence of the death of the cell.

The dye then penetrates into the protoplasm of the cell and produces the nuclear staining, which for vital dyes is the typical histological phenomenon ofthe death of the cell.

The animal must live for some hours after the injection, if we wish to observe distinctly the alterations mentioned above.

For dogs there are other proportions. Goldmann explains the fact that, after application via the liquid of small (not lethal) doses of trypan blue, there is a vital staining of the nerve cells, in consequence of special receptors in those cells. He supposes that the dye does penetrate into the ganglion cell but is there taken up instantly and rendered innocuous by its joining certain cell-granules.

„Mit andern Worten besitzt die Ganglienzelle des Hundes in ihren färbbaren Granulis, Schützstoffe, welche das Eindringen des Farbstoffes an den „Lebenskern" verhüten."

After application of very large doses this defending function disappears and the general toxical phenomena arise.

According to Goldmann, the Choroid plexus defends the nervous tissue against the noxuous influence of the poisonous trypan blue by the specific function of its cells.

„Wird diese Hemmung des Plexus vermittels der Lumbalinjektion umgangen, dann ist das gesamte Nervensystem der Einwirkung unserer Farbstoffe preisgegeben". (p. 45.)

This is, very concisely, the principal point of Goldmann's interesting publication. In his work all kind of experiences are communicated on the course of the cerebrospinal fluid, etc.

In the following chapters I shall make use of them as much as possible.

The experiments described above speedily attracted attention, and it was Mott in particular who pointed out the great interest of these investigations especially in connection with the question of how to apply the antisyphilitic drugs in syphilis or parasyphilis of the nervous system.

In Holland van Hasselt was the first who repeated those experiments in the laboratory at the „Valerius-kliniek" at Amsterdam.

He obtained nearly the same results as his predecessor, but objected from the first to the theory that the choroid plexus was the physiological protecting membrane of the central nervous system.

Many other investigators, attracted to these experiments by their interest in the salvarsantherapy of nervous diseases, repeated them and used them as the basis of their therapeutic trials with paresis and tabes.

Whether they all regard the results of their injections with sufficient criticism will appear in the following chapters.

Especially Marinesco and Minea (in 1914) based upon the fact that after endocranial subarachnoidal injection of trypan blue the whole convexity is stained at the side of the injection, a therapy of paresis with endocranial salvarsan-injections.

Also Robertson and Klausner on the ground of these experiments recommended the intraspinal treatment with salvarsan for syphilitic nervous diseases.

Lately Lafora quoted again Goldmann's experiments and at the same time recommends the intraspinal treatment.

Fortunately criticism is not lacking from very different sides.

Rachmanow has studied particularly the distribution of trypan blue in and around the nervous system after intravenous application.

He obtained the same results as Goldmann.

On examination of experimental lesions of the central nervous system with the aid of vital dyes, it appeared that within a few days very numerous, vitally stained cells arose in the wound and its neighbourhood („ruhende Wanderzellen").

Two weeks after the lesion he found few blue granules in the gliacells in the neighbourhood of the wound, whilst two weeks later granules were to be seen in the ganglion-cells near the wound.

Rachmanow now asks how the defending function of the choroid plexus is to be made conformable to the staining symptoms of the nervous elements under pathological conditions and answers this question by denying the defending function and assuming as the principal factor in the process of staining a definite chemical affinity.

Lewandowsky, a severe critic, also attacks Goldmann's conclusions.

In his opinion, based on his own strychnine-experiments, there is no proof that substances, which penetrate into the cerebrospinal fluid, pass the plexus. He found, with strychnine intoxication via the blood, that the poison was also in the fluid of the lumbal part of the spinal cord, though the dura was ligated in its cervical part.

He therefore assumes that the substances which pass from the blood into the cerebrospinal fluid have not to follow the route of the plexus, but pass through the walls of the blood-vessels.

Ariëns Kappers neither considers the choroid plexus the only protection of the nervous system, as most vessels enter into it by other paths than the plexus.

He assumes that the vessels of the nervous system must also be surrounded by a membrane through which trypan blue cannot penetrate. He has here in view the perivascular glia.

The view of Lewandowsky, namely, that it is the walls of the capillaries, which prevent the entrance of the dyes, would require the capillary-endothelium in the central nervous system to differ metabolically from that of the other organs, where it does allow trypan blue to pass.

In particular do the investigations of Dewey throw considerable doubt upon Goldmann's results. She too believes that the plexus plays a part as a defending membrane of the central nervous system, but asks herself how it can be possible that the filtering process by the cuboidal epithelial cells of the choroid plexus protects the entire brain against injurious substances from the blood-plasma, when other vessels besides the choroidal arteries (in fact the larger number of the cerebral vessels) pass directly into the brain substance.

She could not find toxical symptoms after intraspinal injection of trypan blue, and ascribes the results of Goldmann to mechanical and physical factors (by direct lesions of the extremely sensitive nervous tissue.)

One fact in favour of this view is that she saw the most serious consequences after injections with aqua destillata.

She then examined the staining reaction of intraspinally injected trypan blue on the cells of the meninges and nervous system.

As "positive", she marks the cases where the cells, particularly the cells of the connective tissue are stained vitally, whilst she marks as "negative" the cases in which diffusely stained cells are absent.

Those rabbits which were killed when serious symptoms arose and persisted for several hours were nearly all negative cases. On the other hand in instances where the animals, presenting grave symptoms, were allowed to live and to recover, and subsequently again injected with trypanblue without any serious effects, the results were generally positive. Only in two of the negative cases was intense staining of nervous cells of the cord observed, comparable to that described by Goldmann.

It was due to the direct injection of the staining solution into the nervous tissue.

Tilney injected trypan blue intraspinally into cats and allowed them to live for 24 hours. The dura mater was intensely stained, the brain stained diffusely.

Microscopically he found that the septa of the pia extending into the brain and cord were stained, also the walls of the blood-vessels.

The ganglion-cells and nervous fibres were not stained.

On account of the above mentioned contradictions in medical literature and in view of the eminent importance which this question bears to the intraspinal application of salvarsan, it seemed to be necessary to repeat Goldmann's experiments critically and at the same time to extend them.

Another proof of the importance of Goldmann's investigations is the fact that they have drawn attention to the pathology of the choroid plexus

Haeckel had (in 1859) given a description of some pathological changes in the structure of the plexuses, which was followed by analogous investigations by Imamura, Findlay, Pfeiffer, and others.

Tumours of the plexus were mentioned by Bylchowski, and others.

Von Monakow was the first who tried to find in the changes of the plexus the cause of definite symptoms.

He saw that in embryos, even before the fore brain had attained a certain state of development, the suprarenal gland and the plexus were important organs.

Since in the new-born child the „cry" is the first expression of affect life, and since we know that in the adult the instincts and feelings have a more intense function than the intellectual qualities, Monakow considers the internal glands as „den Mutterboden für unsere Gefühle und Instincte." (p. 365).

He ascribes to the plexus a protective function, and believes that it is capable of destroying toxical substances, which circulate in the organism.

Von Monakow believes that in hystery and neuroses, the activity of the plexus is altered, just as in general paresis and dementia praecox.

Especially in the latter disease are the instincts changed to a pathological degree. And we may therefore expect that the tissues of the plexus, etc. are changed morphologically.

Kitabayashi studied the plexus in Schizophreny and observed hyperaemia, degenerative changes etc.

Perhaps the study of pathologic changes of the choroid plexus, will afterwards give us new insight into he symptomatology of many psychical diseases.

CHAPTER III.

On the intraspinal treatment as a therapeutic method.

It follows from the preceding chapters that, according to many authors, a direct communication exists between the cerebrospinal fluid and the perineuronal spaces.

Considering the hypothesis that the plexus is a protecting membrane, such communications made the intraspinal method a rational one. The demonstration bij Lewandowsky of the difference in action between intravenously and intraspinally applied drugs, was a new plea in favour of this method.

He proved that the difference in pharmaco-dynamical action could not be caused by a difference in quantity of the applied drugs, but that there was also a qualitative difference of action Strychnine for example caused strong spasms after intravenous injection, whereas after direct application on the spinal cord symptoms of sensory excitation arose.

Morphium acted in an analogous way.

Adrenalin, after intraspinal injection caused spasmic contractions of the hind-legs, together with a quick tremor, whereas intravenous application did not cause any symptoms from the side of the nervous system. The action of anorganic salts after intraspinal injection proved to be independent of their anions. Sodium bromide was harmless, potassium bromide caused paralysis, as did potassium iodide. Sodium iodide did not raise any symptoms. Intravenous injections had no such influence on the nervous system.

Lewandowsky now formulated the difference between the action of intraspinal and intravenous injection in the following way:

After intraspinal injection:

1. The substances act in an acute way.
2. They act in small doses.
3. There is often a difference in the toxicological symptoms.
4. Some drugs cause symptoms only after direct application intraspinally.

In this chapter I will endeavour to give a description of the results of this treatment, especially in lues nervosa, whilst communications will also be made on other corresponding therapeutic methods.

Jacob, continuing the experiments of Quincke, examined in 1898 the possibility of introducing drugs into the arachnoid spaces.

He was able to inject large quantities of salt-solution into the arachnoid space in men without observing any bad symptoms.

He intended, by this method, to wash out the arachnoid space with desinfectants (in case of meningitis), or to apply drugs intraspinally. Solutions of potassium iodide were the first he made use of.

Into the arachnoid space of dogs 25 c.c. of a 4 % solution were injected, which was followed by paralysis or intense pains.

In 1900 he applied his experiences clinically to men, in cases of tetanus, neurosyphilis, etc.

Thus he obtained positive results in one case of tetanus, whilst an infusion of strongly diluted solutions of sodium iodide in three cases of genuine syphilis of the nervous system, produced some improvement. At the same time Bier had applied the „Dural-infusion" as a new method to obtain anaesthesia, After intraspinal injection of $^1/_2$ c.M^3. of 0.81 % solution of cocain the lower part of the body up to the mammae could be made analgetic.

Sicard (1899) compared experimentally the action of tetano-antitoxin after injection by the intravenous, intraspinal or intracerebral way. It proved that the last method of treatment was the most efficacious whilst the intraspinal way also yielded good results, provided large doses were injected.

In man the results were negative under similar circumstances. nor did the injection of solutions of potassium iodide answer the expectations. Blumenthal and Jacob wrote in 1898: „Auf Grund all' dieser Ergebnisse glauben wir zu dem Schlusse berechtigt zu sein, dass das Tetanusgift z. Z. des Ausbruchs der Tetanuserscheinungen im Centralnervensystem bereits so fest verankert ist, dass es auch mit Hilfe der Duralinfusion hieraus nicht mehr entfernt werden kann."

v. Leyden obtained improvement in one case of puerperal tetanus.

It may be stated that, especially in America, good results have recently been mentioned from the combined treatment of tetanus with subcutaneous and intraspinal injections of tetano-antitoxin.

Also Lewandowsky pleaded warmly for the intraspinal method, though in his first publication on these subjects he criticized Jacob's results in lues of the nervous system.

The fact that paralysis, etc. showed a tendency to improve within a few hours after the injection, urged him to exclude the usual pharmacological action of iodium.

From what has been mentioned above, it will be seen that intraspinal treatment as a therapeutic method has not been able to resist the attacks of criticism: only the sacral-anaesthesia gives positive results and has been perfected into a common routine-method. (Jonnesco.)

In some diseases the intraspinal treatment is still used:

In meningitis antisera are injected, just as methylen blue in some cases of myelitis and meningitis. (Marinesco).

Nobécourt and Darré treat polyomyelitis by introducing normal serum into the arachnoid space, whilst Flexner makes use of antiserum in the same way.

The results are not satisfactory however. The intraspinal calcium therapy in epilepsy has equally failled. (Obregia et Urechia).

The combating of infectious processes in the arachnoid space by injecting antiseptics has been recommended by Franca and Wolf.

The results were not encouraging; in most cases more influence was to be ascribed to the repeated punctures thans to the action of the desinfectant.

Alford tried to improve these results by irrigating the arachnoid space (as was done in 1896 by Hill, in corpses).

He made a laminectomy in the lumbar region, and after this a trephine-opening in the regio parietalis cranii.

An incision was made in the dura cerebralis and a rubber tube introduced. The injection was made intraspinally.

The irrigatio succeeded. Injected antiseptics flowed out of the rubber tube and the irrigation of the arachnoid space with solutions of boric acid, permanganate of potassium, etc, did not prove to have any unfavourable influence upon the nervous system of animals.

Wegefarth and Essick, on the contrary, were able to demonstrate lesions of the central nervous system caused by antiseptic fluids.

In the chapter: „Critical remarks upon intraspinal treatment", I will say more about these investigations.

The intraspinal treatment of syphilitic nervous diseases has, in contradistinction with the intraspinal therapy of other nervous diseases, become a common method with many neurologists, though its results may not be called brilliant. (Wichura.)

As has been mentioned above, this therapeutic method is based upon the experiments of Goldmann and the communications of Mott and Obersteiner on the intraneural (pericellular) lymph-spaces.

As has already been said the latter writers accept a canalicular system running through the nervous system from the arachnoid space, via Virchow-Robin's sheath to the periganglionic spaces.

A drug, introduced into the cerebrospinal fluid, thus finds its way easily to the ganglion-cells by following these natural pathways. Neither the critical remarks of Nissl, nor the studies of Held on the neuroglia, led to the abondoning of this standpoint so that the intraspinal treatment, remained a routine-method.

Especially the discovery of Noguchi (1912), who was able to demonstrate the spirochaeta in the nervous tissue of patients from tabes or paresis (studies continued by Levaditi, Marie, Jahnel, etc.), was a new stimulus in this direction, most neurologists being now convinced that the spirochaeta is the direct cause of the so-called metasyphilitic nervous diseases.

Since it is known, that salvarsan cannot be indicated in the cerebrospinal fluid after intravenous injection, the consequence of these considerations was to apply neosalvarsan endolumbally.

On account of Quincke's experiments, it was supposed that after intraspinal injection the drug would spread over the whole nervous system. In the following pages I will describe successively the methods used in this treatment, viz.

1°) the intraspinal method.

2°) the intracranial method.

3°) the intracerebral method.

I. The introduction of salvarsan into the cerebrospinal fluid by the intraspinal method can be made in different ways.

a) injection of solutions of salvarsan in aqua.

b) injection of solutions of salvarsan in serum.

a) Injections of salvarsan solutions.

Wechselmann, was the first who injected large doses of neosalvarsan. He even applied 6 m.G. pro dosis, without observing bad consequences. Gennerich especially has worked out this method.

He avoids large doses and injects highly diluted solutions. He makes a lumbar puncture, and after the outlow of 30 c.c. of cerebrospinal fluid, mixes a part of this fluid with a solution of neosalvarsan, and injects this mixture into the arachnoid space with the aid of a burette.

In tabes Gennerich begins the treatment with very small doses: 0.67 m.Gr. of neosalvarsan.

In paresis he mounts, however, to 2.25 m.Gr. per injection.

Between the injections he allows intervals of 2 to 3 weeks. He does not mention any bad results; as to the clinical results he is rather optimistic.

Von Schubert makes a solution of neosalvarsan in the punctured liquid itself. He gives 3 m.Gr. pro dosis and sometimes observes little improvement.

In judging the results, we are confronted by two difficulties:

1) The treatment of lues nervosa is nearly always a com-

bined one, so that it is impossible to ascribe the results to the intraspinal injection only.

whilst 2) The results obtained by most investigators are generally put in a too favourable light.

Good results may be recorded of the intraspinal treatment of meningo-recidives. Of the new salvarsan preparations, silver salvarsan-natrium has been applied intraspinally. On the results no final judgment can yet be pronounced.

b) Injections of salvarsanized Serum.

This is either a solution of salvarsan in serum or is the serum of patients (or animals) who are being treated with salvarsan.

Marinesco was the first to introduce this method.

Thus he treated (in 1914) 21 patients of paralysis with serum, salvarsanized „in vitro", and in 7 cases obtained improvement of the psychic state.

Swift and Ellis have made use of this method on a large scale, and many American neurologists have put it into practice.

One hour after intravenous injection of salvarsan, a venasection is made, and 40 c.M^3. of blood allowed to flow into a centrifugal tube.

The blood is allowed to coagulate and the serum separated from the coagulum. The next day 12 c.c. of the serum is diluted with 18 c.M^3. of normal saline solution.

This solution (serum and physiological salt-solution) is heated to 56° and kept at this temperature for half an hour (as based on the fact, that, according to Stühmer, heating of the serum increases its action).

A lumbar puncture is made, and from 5 to 15 c.c. of cerebrospinal fluid is drawn off till the pressure falls to about 30 m.M. of mercury. Thirty cubic centimeters of the warmed serum are then injected into the arachnoid space.

Swift and Ellis preferred to use the serum of the patient himself. Their method is founded especially on the experiments of Gonder, who succeeded in indicating that salvarsan itself does not kill the spirochetes, unless activated in some way by serum (investigations of Nierenstein also plead for this idea).

Swift and Ellis obtain good results: new investigations, however, may show whether those results are not to be obtained equally with normal serum.

Spencer applied the method with tabetics: the lancinating pains sometimes proved to disappear 36-48 hours after the injection.

This gives rise the question whether this is based only on a spirochetocidal action or on a physical action.

Why the striking character of the results? It is at once evident that the amount of spirocheticidal substance in a few cubic centimeters of serum taken from the body of another patient who had received the usual intravenous salvarsantherapy must be very small indeed, considering its amount of arsenic.

Notable in this respect is the third case of Spencer, where there was no arsenic at all in the so-called salvarsanized serum. Nevertheless the injection had made the lancinating pains cease for a week.

Nearly all students, (Schrottenbach, etc.) who have applied this treatment, mention good results; they consider the most striking, suggestive result is the almost immediate diminution in the number of pathological cells in the cerebrospinal fluid. The globulin reaction diminishes, positive Wassermann of the cerebrospinal fluid become negative, etc.

But, as the same results have been obtained in many cases by a simple intravenous treatment, it is very difficult to ascribe the excellent results to the intraspinal treatment only.

According to Stühmer, the striking results obtained with the application of salvarsanized serum is due only to the quantity of salvarsan dissolved in it. The spirocheticidal action of those sera runs parallel with their amount of salvarsan (as demonstrated by the reaction of Ehrlich-Bertheim).

Gurari, as well as Gradwohl et Levaditi records excellent results: the latter even saw the lost tendon-reflexes in tabes re-appear.

Lafora has recently recommended warmly the „in vitro" method of preparing salvarsanized serum. He makes a solution of 150 m.Gr. of neosalvarsan in 5 c.M. aq. bidestillata, and mixes this solution with normal serum. He generally injects salvarsan to an amount of 7 m.Gr. intraspinally, and often observes amelioration.

In paresis, intracranial or intraspinal injections of salvarsanized serum only give increasing evidences of amelioration in fresh cases. In tabes especially the subjective symptoms disappear.

In Holland F. Meyers and L. Bouman have applied the method of Swift-Ellis; neither of them, however, has been able to communicate happy results.

In paresis L. Bouman speaks of „disconsolate" results, as does also Sachs. Sicard has obtained better results (in tabes).

He believes that the causal lesion of tabes is localised „au niveau des culs-de-sac arachnoïdo-pie-mériens ganglionnaires."

He intends, therefore, by means of the intraspinal injection, to cause a meningeal reaction, which may free the posterior roots of their infiltrations. He makes use of normal saline or of a highly diluted solution of Hydrargyrum cyanide.

This preparation should cause a more intense meningeal reaction.

Besides this „réaction meningée libératrice", Sicard believes that the „perméabilité méningée" increases, (as occurs in case of aseptic meningitis: see chapter I).

Consequently he recommends this intraspinal treatment to be combined with an energetic attack by intravenous methods. His results are excellent; especially the lancinating pains, as directly caused by the involvement of the posterior roots, quickly disappeared.

Also mercury solutions have been made use of in intraspinal treatment: Page recommended hydrargyrum succinamide, Byrnes sublimate, whilst Mestrezat et Sappey injected electromercurol (colloidal mercury).

According to those authors the action of colloidal mercury must be considered as a a dual one:

1) an action on the meningeal processes (amelioration of the lancinating pains);

2) an action on the sclerotic processes, which are fundamental for tabes, and which are responsable for its cardinal symptoms.

Weichbrodt injected intraspinally in a patient with general paresis, 5 c.M^3. of a I % solution of methylene blue silver.

No advantage was to be seen; the patient remained in his former condition.

After injection of 1 c.M^3. of a 1 % solution, paraplegia followed, accompanied by high fever. Patient died 6 days after the treatment. Cord and meninges proved to be stained intensely blue, whilst the base of the brain and the bottom of the fourth ventricle showed a light-blue colour.

2. THE INTRACRANIAL INJECTION.

As early as 1910, Horsley, after making a trephine-opening, moistened the convexity of the brain in general paresis with a 1: 10000 solution of sublimate.

Marinesco and Minea made a puncture of the skull, and introduced highly diluted solutions of salvarsan into the arachnoid cavity of the brain.

They injected large doses, up to 50 m.Gr., and from time to time saw Jacksonian attacks appear.

In 4 out of 17 cases slight psychical amelioration could be observed. Neurologically no changes could be demonstrated.

Intraventricular injections of salvarsan were applied by Hammond and Sharpe, who considered this method a less dangerous one than the common intracranial way. Ballance and Campbell introduced salvarsanized serum into the lateral ventricles.

Doyen injected subdurally into the cranial cavity „salicylarsénate de mercure". Epileptic attacks followed after the second injection. More rational was the method followed by Levaditi, Marie and Martel. It had been proved from investigations of Levaditi and Muttermilch that in animals, infected with Trypanosomes and treated with salvarsan, the specific immune materials were to be demonstrated in the blood after the disappearance of the circulating parasites.

Consequently the authors intended to make use of a rabbit serum, that has a high amount of spirocheticidal substance. Therefore they applied the serum of syphilitic rabbits, which had recently been treated wich salvarsan. This contained salvarsan ± immune materials. They injected subdurally a mixture of 3 c.c. of rabbit serum and 2 c.c. fluid of the patient. The results obtained were not brilliant.

Bériel et Durand modified this method by substituting the rabbit serum for serum of a patient with secondary syphilis, but got no better results.

From the facts mentioned above it may be demonstrated sufficiently that the results of the intraspinal treatment in tabes or paresis uptill now have been far from brilliant.

Still many authors, however, consider this method as an efficacious one, so that it has been necessary to give a short review of the methods employed.

In the following chapters a critical review will be given of the theoretical basis of these methods, especially because, notwithstanding this way is a dangerous one, there is a general tendency to look for better results in this direction.

3. THE INTRACEREBRAL METHOD.

In the following lines some communications will be made on the intracerebral application of drugs.

The first authors who recommended this method were Roux and Borrel: they injected small doses of tetano-toxin into the brain of rabbits and observed epileptic attacks that lasted for 1-5 days.

On their instigation Biedl and Kraus studied the results of the intracerebral application of bile-acids and observed symptoms of excitation which are not typical of the injected substance however.

Bruno tried to make this method a pharmacodynamical one, though Lewin rejected it a priori on the following grounds:

„Denn da die experimentelle Pharmakologie sich zur Aufgabe macht, die Wirkung chemischer Agentien auf den Organismus zu verfolgen, so musz im allgemeinen die Einführung der Substanz eine derartige sein, dasz es dem Organismus selbst überlassen bleibt, wie sich das Gift auf die einzelnen Organe verteilt, und auf welche Organe die Wirkung sich demgemäsz erstreckt....

Durch die direkte Injection des Giftes in die Gehirnsubstanz, umgehen wir aber diese Verteilung des Giftes vom Blute aus und schaffen in sehr giftempfindlichen Elementen, eine so hohe Koncentration des Giftes, wie dieselbe durch Verteilung vom Blute aus, vielleicht nie zustande kommen kann."

Nevertheless Bruno applied different poisons.

Morphium caused a quick tremor, followed by motor excitation and increased reflex excitability. (The rabbit made movements of flight).

About 3—5 minutes later, clonic and tonic convulsions were to be seen in all the muscles, interupted by violent rolling movements.

Death followed in one to 3 hours under symptoms of respiratory-paralysis and paralysis of all other muscles.

The injection of a 4% solution of ferrycyannatrium had the same results. Small quantities of normal saline could not produce this action.

From those experiments we may conclude, that the intracerebral injection of different poisons may produce analogous symptoms.

Probably this depends upon a direct local excitation of certain brain-centres.

As will be communicated afterwards in my „Own Experiments", also physical changes, (trauma, etc.) caused in the nervous system, are capable of producing analogous symptoms.

CHAPTER IV.

My own Experiments.

In this chapter I intend to give, with the aid of protocols and pictures, a survey of my own experiments made with trypan blue upon rabbits. The following series of experiments will be discussed:

I. Intravenous injections
II. Endolumbal „
III. Endocranial „
IV. Intracerebral „
V. Intravenous injections after partial removal of the plexus
VI. Intravenous injections after section of the spinal cord.
VII. Application of alcohol, etc. in order to make the plexus permeable.

I. Intravenous injections.

I always used a $^1/_2\%$ solution of trypan blue (Hoechst).

Large doses were intravenously applied: e.g. 30 c.c. They were injected into the marginal ear-vein. The animals were not etherized, since the injection was practically painless. As an example of an intravenous injection the following protocol may be mentioned:

Rabbit EE:

In several tempi ± 23 c.c. of trypan blue are applied. Very soon (in about half an hour) the visible mucous membranes, especially the conjunctivae, become distinctly blue. No injections are made for a week: the animal emaciates but continues eating and is lively. On the 28th of January 1920 another 8 c.c. of the dye are injected intravenously.

By accident an air-bubble gets into the syringe; suddenly the animal succumbs with slight convulsions.

Immediate autopsy: the skin of the ears is blue; also the conjunctivae bulbi. The subcutaneous conective tissue is distinctly blue. Opening of the vertebral canal and skull: strong blue staining of the dura; the pia is light blue.

The nervous system is colourless.

Chest: heart: light blue

lungs: collapsed, no bleedings. Blue tint.

Abdomen: Diaphragm: the tendinious part is distinctly blue.

stomach — blue
bowels — light blue
liver — very dark blue
kidneys — dark blue.

Peritonaeum and mesenterium — dark blue, especially along the vessels.

Suprarenal gland — not stained.

The blood is violet; a blood smear on glass is made and fixed 20 minutes in absolute aethylalcohol.

Some of the leucocytes in the preparation show a blue tint.

Liver, kidneys, heart, spleen, peritonaeum, bowels are microscopically examined (in frozen sections).

Microscopical examination of the organs.

Central nervous system: the tissue is fixed in a 10% solution of formaldehyd. Frozen sections are made of 10 microns and examined in glycerine (under a glass cover).

In the nervous system itself blue staining can nowhere be indicated. In the pia, which surrounds the sections, oblong or round cells are to be seen, full of fine granules.

The pia is studied separately: the granulated cells lie in clumps togethed. They are chiefly round; the nucleus is not visible. Some show offschoots. Also fine fibres of the connective tissue are coloured here and there in the neighbourhood of the cells. The choroid plexus which microscopically appeared to be distinctly blue, was studied as a whole in glycerine.

Its epithelium cells are distinctly blue, which blue staining is founded upon an accumulation of coloured granules. The nucleus is never stained. If the glass cover is pressed, secrete drops coms free from the epithelia into the glycerine; these are never blue.

The interstitial tissue of the plexus villi is again distinctly stained, especially by the presence of groups of round, blue granulated cells.

In the liver the blue staining is limited to the capsule and groups of interstitial, evidently Kupffer's cells.

In the kidneys the vessels are distinctly coloured: the endothelium is colourless. The blue staining is principally localized in the membrana elastica interna.

Also groups of epithelia in the tubuli contorti show a granulated staining.

In the heart the muscular tissue is colourless, but definite interstitial cells are blue.

RABBIT FF.

Intravenous injection of 10 c.c. trypan blue.

After one hour blue staining of the visible mucous membranes. After three days the animal is killed.

Light blue staining of the meninges and plexus.

The organs are stained in the usual way. (see above).

Microscopical examination gives analogous results as with rabbit EE.

Summarizing, it is proved from the above protocols, that:

1) Definite cells are vitally stained.
2) These cells are: a) the diffusely spread pyrrolcells and interstitial cells;
   b) the epithelial cells of the plexus, kidney and hypophysis.
3) The cells mentioned under b) have together a secretory function.
4) Organs which do not contain cells agreeing with those mentioned sub 2), are not coloured blue.
   Among those the central nervous system and retina muse be mentioned.

Consequently an animal intravenously injected with trypan blue in large quantities remains neurologically intact and shows after autopsy no staining of the nervous parenchym. (This is the result of the above protocols as well as from experiments made upon four other rabbits).

INTRA ARACHNOIDAL INJECTIONS.

What will take place if dye is introduced into the subarachnoid cavity?

This may be done:

a. Endolumbally.

b. Endocranially.

II. Second series of Experiments.

The results of the endolmbal injection of a vital dye may be considered from different standpoints.

We may study the spread of the trypan blue upon the whole surface of the nervous system, its penetration into the nervous system and the physiological reactions of the animal. We may also devote our attention to the course of the lymph paths; to the structure of the perivascular lymph spaces, to the pathway of the cerebrospinal fluid, and we may pay attention to the choroid plexus, to the pia and the protecting membranes (limitans gliae) of the nervous system.

As to the technical side, the following may be mentioned.

In his first experiments Goldmann laid bare the spinal cord. His operation, however, proved to be too radical. Also the method of Klose and Vogt (laying bare the spinal colum before the punction) was abandoned by him, whilst the free puncture directly into the arachnoid cavity was practised as the simplest method. Especially younger animals were used. The animal was narcotized and the puncture made in the strongly bent spinal column. Invariably, when the injection was considered as succesfull, a swift contraction in the lower extremities followed. The injected quantity was with rabbits never more than 0.5 c.c. of a $^1/_2$ % solution of trypan blue.

The method of Goldmann, called „free puncture", has been abandoned by me as not being sufficiently reliable. At the height of the lumbal part of the cord an incision was made into the well narcotized animal. The wound was kept open, so that a wide field for operation arose. With a sharp pair of scissors 2 or 3 processi spinosi were cut off, whilst the arches of the vertebrae were cut away. In this way the dura was laid bare. In it an opening was made, and the dye was prudently injected at first under low pressure with a common injection-needle, which I soon replaced by another needle, in order to prevent eventual lesion of the pia or nervous tissue. For this purpose a common injection-needle was slightly curved and the point ground off. Of the hollow cylinder which was formed in this way, the edges were made round.

Now lesions could be precluded absolutely.

That this method is entirely faultless I cannot maintain. But

I am convinced that it is more certain than the blind method of the free injection. A great drawback is the profuse and even letal bleeding which occurs sometimes.

A $^1/_2$ % solution of trypan blue was always applied; the quantity to be injected varied, however, but was generally not more than 1 à $1^1/_2$ c.c.

The first thing that struck me was that the symptoms of intoxication of the nervous system, described by Goldmann, were absent.

Goldmann describes these symptoms as follows (p. 32):

„Zunächst lagen die hinteren Extremitäten des Tieren, ausgestreckt, wie gelähmt da. Dann fingen in denselben klonischen Zuckungen an, die sich rasch über den Rumpf nach den vorderen Extremitäten ausbreiteten. Das Tier erwachte häufig mit einem Schrei aus der Narkose, versuchte sich auf die Füsze zu stellen, und in rasender Flucht sich fort zu bewegen. Stiesz es an einem Gegenstand an, so machte es halt, der Kopf wurde durch einen opisthotonischen Krampf steif nach hinten oder umgekehrt durch einen emprothotonischen nach vorn geworfen, u.s.w."

I could never observe these symptoms with my endolumbal injections. So for instance I wrote in the protocols about the endolumbal injections as follows:

RABBIT A: the animal lies quiet during and after the injection.

RABBIT H after the injection shows a paralysis of the hindlegs from which it does not recover; no tonic or clonic spasms, however, occur.

The same may be said of RABBITS L, N and R.

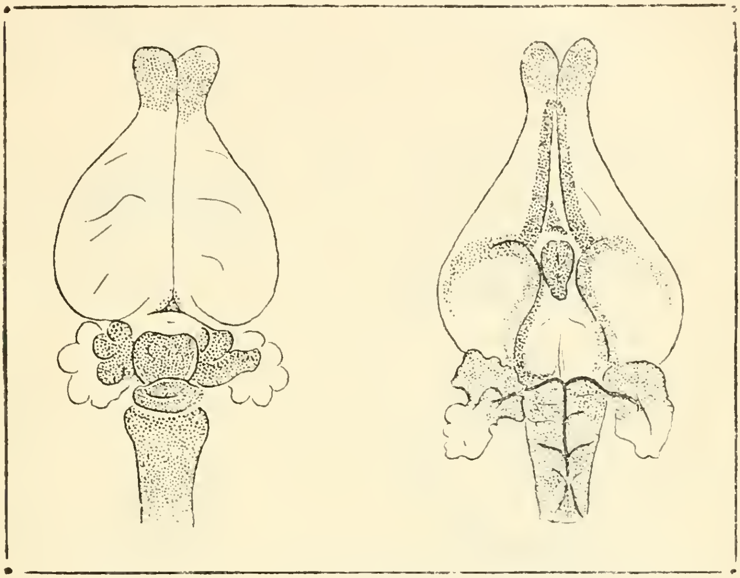
The last animal succumbs 11 hours after the operation without having shown the least symptoms of irritation.

Animal BB dies during the injection from sudden paralysis of the respiration. In this connection I may remark that whilst I exposed the spinal cord a very profuse bleeding took place, which could hardly be stopped.

Usually the injected animals were killed after 12-16 hours. Immediately the nervous system was laid bare and the animal was put into a basin with a 10 % solution of formaldehyd or Joris' liquid. The next day, if sufficiently hardened, the nervous system was prudently prepared and microscopically studied in sections, whilst schematic drawings were made of the spread of the stain.

In most animals, endolumbally injected, there existed a distinct blue staining on the surface of the whole spinal cord (pia). Also the medulla oblongata showed this colouring, The basis cerebri was stained in a very specific and typical way: The ventral aspect of the brain of RABBIT L, e.g. shows the staining in the following way. (Fig. 3.)

Fig. 3.



Rabbit L.

Endolumbal injection of Trypan blue. The dotted parts are stained blue.

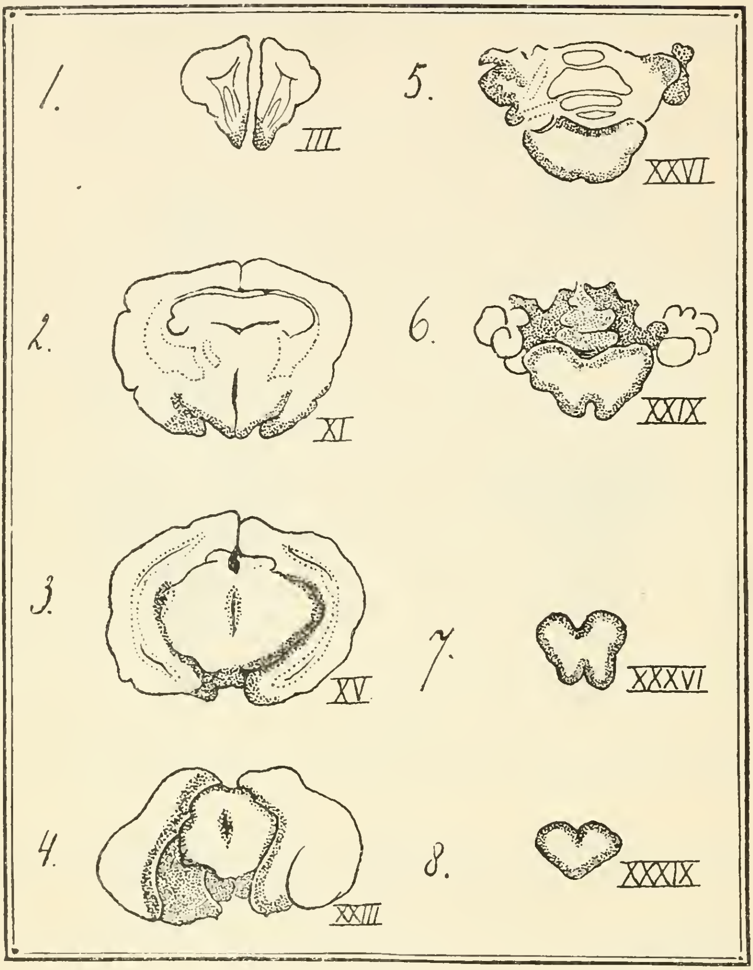
Blue stained are: medulla oblongata, ventral part of
the cerebellum (the flocculus excepted).
pons.
hypophysis.
chiasma and Tr. optici.
tract. olfact. and bulb. olfactorium.
part of the temporal lobes.

In the dorsal part the following parts appear to be coloured:
bulb. olfactorius.
cerebellum (the flocculus excepted).
pineal body.
medulla oblongata.

In all my cases the convexity of the hemispheres is free of dye. Even along the vessels no traces are visible.

In sections (microscopical) the distribution of the dye is as follows: 1)

Fig. 4.



Rabbit L.
Endolumbal Injection of Trypan blue. Frontal Sections.
The dotted parts are light blue.

RABBIT L.

1. Rhinencephalon (corresponding to plate III) (fig. 4). Blue staining of the pia at the basis of the Rhinencephalon in the neighbourhood of the tractus olfactorius.

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1) It may be mentioned that of the brains of Rabbits L and H etc. vertical sections have been made (after hardening in formaldehyd) which were studied immediately. In the description of these sections I will follow the atlas of Winkler and Potter.

2. Section of the Telencephalon and the proximal end of the thalamus opticus (c.f. plate XI): Blue staining of the pia of the rhinencephalon, almost as far as the fissura rhinica. Also the infundibulum is coloured as far as concerns the surrounding membranes.

3. Section through Telencephalon and Diencephalon (plate XV). Blue staining of the infundibulum and of the pia between tel- and diencephalon. Also in the aquaduct is blue staining visible.

4. Section through the mesencephalon (at the height of the corp. quadr. anticus) (plate XXIII): Again blue staining of the pia, which forms the border between mes-and telencephalon. The occipital poles of the telencephalon are both light blue (principally the basal part). Also the ependyma of the aquaduct shows blue staining.

5. Section through the most distal part of the mesencephalon and the cerebellum (plate XXVI): Blue staining in the ventriculus IV and the pia septae, which penetrate between the cerebellar lamellae.

7. The medulla oblongata is also wholly surrounded by blue pia.

From the above description we may conclude that the blue dye has been taken up after endolumbal injection by the pia at the basis of the brain, the cerebellum, the medulla oblongata and the spinal cord. The dye has also penetrated into the third and fourth ventricle and the aquaductus Sylvii. We cannot find any dye in the lateral ventricles however. The convexity of the brain is also quite colourless.

The microscopical examination gave important information. I expected, in accordance with Goldmann, diffuse blue staining of the leptomeninges and of the lamellae of the cord. Also the glia cells should be stained as well as the ganglion cells.

THE MICROSCOPICAL EXAMINATION was made in the following way:

I made frozen section of 10 microns. In order to prevent them being discoloured by alcohol, the sections were put into glycerine. A glass cover surrounded by an edge of varnish was put on them.

I studied sections of the spinal cord near the place of injection, (thoracic region), above it, through the cervical region and the medulla oblongata.

Examination of the spinal cord of Rabbit L at the level of the injection:

Blue staining of the pia and its septa. Staining of the glia cells and ganglion cells, the nucleus and offshoots being distinctly blue. Staining of the walls of the vessels.

It is important, in connection with these facts, to notice that the spinal cord showed a wound in which bleeding had occurred, apparently in consequence of an accidental lesion.

The blue staining of the nervous tissue near this lesion, was a more intense one than in more distant parts, in so far as the ganglion cells of the neighbouring cornu anterior were more intensely stained than those of the cornu anterior, which was not wounded.

Section through the spinal cord at 5 c.M. distance of the injection place:

The pia is light blue, like the membrana elastica interna of the arteries, penetrating into the cord.

The central canal is maximally widened and filled with a blue homogeneous mass.

The ependyma cells, in normal conditions generally lining the central canal, are partly pushed off. Some which have remained, are diffusely stained (apparently not a vital staining).

Groups of ganglion cells, in the immediate neighbourhood of the widened canalis centralis, are distinctly stained. The cells of the cornua anteriora and posteriora, however, are colourless.

Sections through the cervical region, etc.: the central canal is widened, vital staining of the pia cells. The ganglion cells are all colourless (just as in medulla oblongata, pons, etc.), except those which lie in the tissue surrounding the widened central canal.

Vital staining of the ganglion cells (in the sense of Goldmann) were never be observed.

The above-mentioned facts equally hold good for my other preparations, e.g.

Rabbit N: Diffuse staining of the ganglion cells at the level of the injection.

Sections of the cord, at some distance from this, show another image: intense granular staining of pia- and pyrrhol-cells. The parenchym is colourless.

ENDOLUMBAL INJECTION.

INTRASPINAL INJECTION	SPREAD OF THE DYE	SYMPTOMS OF IRRITATION (GOLDMANN)	STATE OF THE SPINALCORD AT THE PLACE OF INJECTION	AT A HIGHER LEVEL	CHOROID PLEXUS	EPENDYMA
A	up to the first cervical segment	death by narcosis	—	—	unstained	blue
H	normal type	paraplegia of the hind legs. Death 24 hours afther the injection	stained diffusely	nervous tissue is colourless. the pia is blue.	unstained	blue
L	normal type	paraplegia of the hind legs. Killed 8 hours after the injection	stained diffusely	nervous tissue is colourless. canalis centr. and its surrounding are blue.	unstained	blue
N	light blue staining of the base of the brain	paraplegia Killed 12 h. after the injection	stained diffusely	nervous tissue colourless.	unstained	blue
R	normal type	paraplegia Death 10 h. after the injection	staining of pia and canalis centralis	nervous tissue is colourless. pia and central canal are blue	unstained	blue
BB	normal type	Died from bleeding 10 min. after the injection (No lesion of the nervous tissue)	staining of pia and canalis centralis	nervous tissue is colourless. pia and central canal are blue	unstained	blue

Summarizing, we may therefore state, that after endolumbal injection of trypan blue:

1) no symptoms of irritation (in the sense of Goldmann) arise.
2) a granular staining of the pia, surrounding the spinal cord and at the base of the brain, occurs.
3) a staining of the ependyma appears, lining the ventricles.

4) a diffuse staining occurs in the nervous tissue of the spinal cord near the injection, provided the nervous tissue is damaged in some way, or the injection has been made into the nervous tissue itself.
5) no staining may be observed in higher parts of the spinal cord, unless, owing to the high injection-pressure, widening of the canalis centralis with consecutive lesion of the ependyma cells appears. Then, round the central canal, blue staining of the ganglion cells may be observed.

The result of the second series of my experiments may be given in the scheme (see page 53).

From the above it appears that Goldmann's results are doubtful.

Other results of the second series of Experiments:

In the first place we must draw attention to the remarkable spread of the dye along the cord, the basis cerebri, etc.

In chapter I, I already drew attention to this, and at the same time pointed out the relations between this spread and that of some meningitic processes.

The thought occurred that the basal spread of the dye might be founded upon the action of gravitation.

I tried to answer this question in an experimental way. My intention was to eliminate gravitation by making the base of the brain not the lowest part of it. (See chapter I.)

RABBIT L.

4—7—'19 10 o'clock a.m. After the usual preparation an incision is made in the skin at the level of the lumbar part of the cord. The wound is kept open and the muscles are laid to the sides. The processi spinosi of 3 or 4 vertebrae are cut away and the dura is carefully exposed.

Endolumbal injection of $^1/_4$ c.c. of a $^1/_2$ % solution of trypan blue.

After the injection the animal is laid on his back with the basis cerebri turned upwards. It did not scream, nor show any motor excitation, nor clonic and tonic contractions. Pulse and respiration are rather slow.

4.30 p. m.: The animal is killed by bleeding.

Immediate autopsy:

Blue staining of the pia of the spinal cord, especially about the place of injection, the dorsal part of the oblongata, the cerebellum, (the flocculi excepted) and the rhinencephalon.

Notwithstanding the reversed position directly after the injection, the basis cerebri is coloured in a typical way, and the convexity of the brain is unstained.

Rabbit N.

11—7—'19 11 o'clock a. m. In order to demonstrate this experiment in a more convincing way, the rabbit is placed in a reversed position during the injection. In this way $1^1/_2$ c.c. of trypan blue are injected endolumbally.

The animal remains in this position with the basis cerebri turned upwards till about 10.15 in the evening, when it is killed.

Blue staining of the pia of the spinal cord, diminishing towards the cerebellum.

The convexity of the hemispheres is quite colourless.

From these experiments I may conclude that gravitation has not any active influence on the distribution of the dye.

Another important question, though already studied by many authors, is that of the path of the cerebrospinal fluid.

In most nervous systems I observed a blue staining of the ependyma lining the ventricles, in some even into the mesencephalon (L. & R.)

This makes it necessary to assume a communication between the cerebrospinal fluid and the ventricle liquid.

The staining of the ependyma was usually a vital one, the nuclei being colourless (unless the ependyma cells were injured).

This pleads for a resorptive function of the ependyma. (see chapter I).

As to the choroid plexus, in rabbit L a vital blue staining of it could be observed. This fact is of importance in connection with Dustin's experiments (Chapter I).

Another point which I can confirm is that of the part acted by the nasal mucosa as a path for the cerebrospinal fluid.

Already Key and Retzius have drawn attention to this fact.

In most rabbits, injected endolumbally, blue staining of the olfactory mucosa was to be seen. This proves that dyes leave the cranial cavity by penetrating into the nasal region.

To this chapter we must add a description of the aspect of the blood-vessels which penetrate into the nervous system.

Goldmann (p. 40) describes the images which he found of the vessels and their walls. Everywhere he found the vessels accompanied by vitally stained cells. Between the nervous system and the wall of the vessel he observed a broad space, bounded by cells most of which have taken up the vital dye. Their nuclei are never coloured. „Es kann gar klein Zweifel unterliegen, dasz wir hier Zellbilder vor uns haben, die den perivaskulären Scheidenraum der Gehirnfäsze begrenzen.... etc." (p. 41). My observations agree with this. I must state, however, that the elastica interna, too, shows a distinct staining. I may draw attention to the results of the intravenous injection of trypan blue. In liver and kidney, for instance, the vessels are also stained and especially the membr. elastica interna is distinctly blue.

Summing up, we conclude from this series of experiments (which agree with results of Käthe Dewey and Tilney), that after endolumbal injection of a solution of $^1/_2$ or 1 % of trypan blue the typical symptoms, as described by Goldmann, do not appear. The animal remains quiet and it sometimes, if the injection took place less carefully or if the shock was too strong, shows a paraparesis of the hind legs which may pass. On microscopical examination of the nervous systems there is seen to be blue staining of the spinal cord, cerebellum, basis cerebri, etc., whilst the convexity of the brain is absolutely colourless, even if the influence of gravitation is reversed. With microscopical examination of the nervous system I got other results than Goldmann. At the place of injection, obviously as a consequence of primary lesion of the nervous tissue or by direct injection of the dye into the nervous tissue, a diffuse staining of all elements (ganglion cells, glia cells, pial septa, bloodvessels) is found. The ependyma cells, too, are blue. The colouring of all these cells is not "vital". If, however, we study parts of the N.S. which are at a certain distance from the place of injection, the ganglion cells prove to be colourless. In the pia vitally stained oblong cells are found (pyrrolcells, klasmatocytes, the number of which increases especially in inflammation of the meninges), whilst the ependyma cells of the central canal also show vital colouring. The septa and vessels are, as at the place of injection, distinctly blue.

The plexus in some cases shows vital staining.

III. Third Series of Experiments.

Endocranial, Intra-arachnoidal injections.

With a number of animals the dye was brought into the arachnoid space of the skull, an operation which was generally an easier and quicker progress than the endolumbal application.

The technical part of the operation is very simple: the skin is cut, the periost is pushed aside and a part of the skull removed down to the dura. The dura was cut and the desired quantity of liquid was injected (ethernarcosis). Some protocols follow.

27—6—'19. Rabbit G.

Ethernarcosis. Section of the skin along the sagital line of the skull. The periost is removed. An opening is made in the skull (at the right side) down to the dura. The dura is cut, so that the pulsating brain becomes visible.

Injection of 1 or $1^1/_2$ c.c. of a $^1/_2$ % solution of trypan blue under the dura, so that the pia is not damaged. The wound is closed. When the animal awakes from the narcosis it shows no abnormalities.

After about 15 minutes the animal shows signs of weakness, and while walking it falls sidewards. If irritated the animal rises and assumes again its normal attitude. This condition of weakness lasts only for 15 minutes.

28—6—'19. The condition is very good, the animal begins to eat again.

30—6—'19. No abnormalities. Eats well and is lively.

At 5 o'clock p.m. the spinal cord is exposed in the usual way at the level of its thoracic region, under ethernarcosis. The pia is lightly blue. The animal is killed by increasing the narcosis and bleeding to death (from the carotides).

Immediately the post mortem is made. The pia of the whole spinal cord is light blue. The pia of the medulla oblongata, as well as that of the cerebellum also shows a distinct staining Hemispheres and rhinencephalon are distinctly blue, so are the cavities of the ethmoid and nose. At the left hemisphere a process of suppuration occurs near the opening in the skull. There the staining is less intense than at other spots. The suppuration also extends over the rest of the nervous system. The sheath of the N. opticus is intensely blue. The colouring of the basis is of the usual type.

Rabbit C. C.: (7—11—'19).

At the left side of the skull a small opening is made in the usual way. One c.c. of trypan blue is injected intra-arachnoidally.

After the narcosis the rabbit does not show any pathological symptoms.

14—11—'19. Again 1 c.c. of trypan blue is injected arachnoidally.

Slight meningitic changes are present (pus of the wound). The animal does not react however with any symptoms.

21—11—'19. The same. During the injection a bleeding occurs.

22—11—'19. The animal gets worse and worse. The bleeding continues. Death.

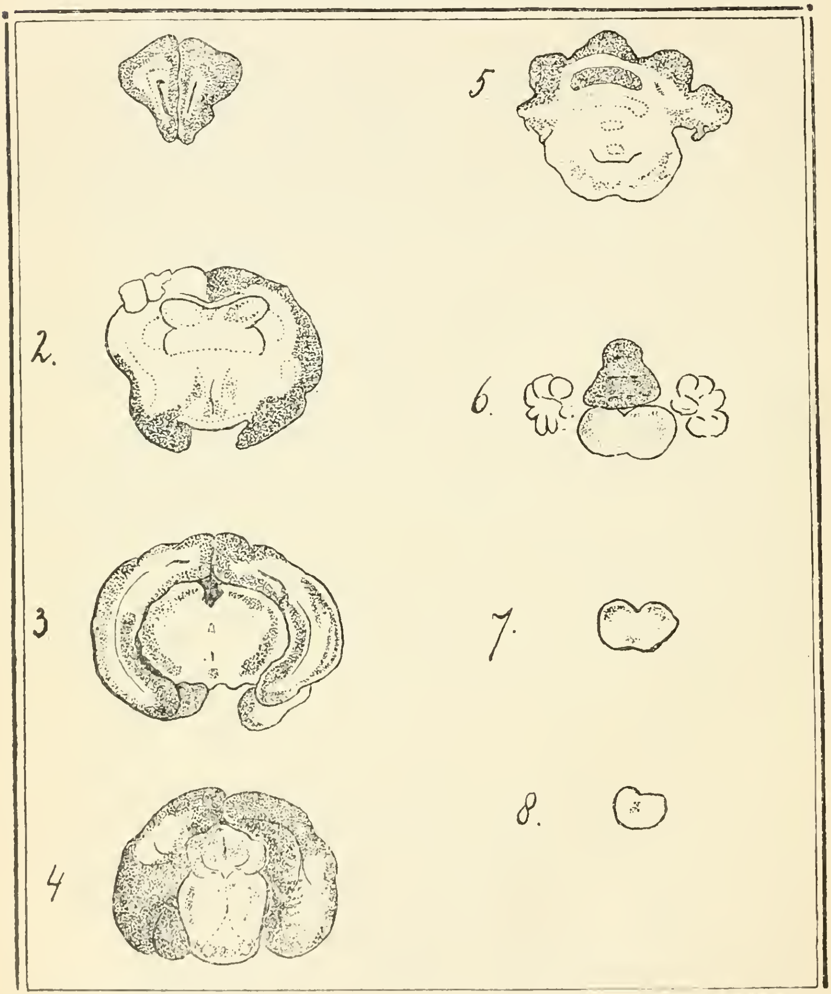
Autopsy: Light blue staining of the pia of spinal cord and hemispheres. The basis cerebri shows the usual staining.

It is again striking, that the symptoms as described by Goldmann and which agree with the symptoms mentioned by Bruno after intracerebral injection, are lacking.

Description of the spread of the dye (studied in frontal sections).

Rabbit G. (see figure 5).

Fig. 5.



Rabbit G.

Endocranial injection of Trypan blue.
The dotted parts are light blue.
(4. The occipital poles of the brain are visible).

fig. 1) Rhinencephalon (III)[1]: a thin edge of a light blue colour round the whole rhinencephalon.

fig. 2) (XI): the rhinencephalon part, here visible, is light blue, as are the surroundings of the third ventricle and the region of the injection place.
(The coloured strip of nervous tissue is about 2 m.M.).

fig. 3) (XV): a thin edge of the periphery of the whole telencephalon, with an edge of the diencephalon are coloured.

fig. 5) (XXVI): blue staining of the intercerebellar septa

Of Rabbit CC. the staining is by far less intens: its spread is similar to that described with the endolumbal injection (Rabbit L e. g. see above).

Microscopical examination:

a) Description of a section through the rhinencephalon near the puncture:

At the place where the N.S. was exposed, a swelling of the meninges is to be seen. In the meshes of this swollen tissue a large number of typical cells is found. They are large, round or oval (of the size of 2 or 3 erythrocytes). Their nucleus is not stained; the protoplasm is filled up with blue granules, of which some are light blue, others very dark.

These granules vary in size.

Also in the deep layers of the brain these cells may be indicated. The number of intracerebral capillaries near the meningitic process has increased. Round them analogous cells are found. but more oblong, and filled with granules. The leucocytes are colourless.

Some ganglion cells near the meningitic process show a diffuse staining. Most of the blue cells observed amidst the nervous tissue, however, are large round ones, as described above. ("Körnchenzellen", pyrrholcells). Below the region of the injection there is a rather serious bleeding, which has probably arisen under influence of the operation.

Round this haemorrhage different macrophages are to be seen, whilst in a small part, situated between the haemorrhage and periphery, there is again a number of diffusely stained ganglion cells.

Also in the part of the rhinencephalon, medially to the ventric. lat., there is a haemorrhage, of the same aspect as that described above.

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[1] The roman ciphers indicate a corresponding plate in „An anatomical Guide to experimental research on rabbits brain." Winkler and Potter.

The blood-vessels show the typical image: the membr. elast. interna is stained light blue. The vessel is surrounded by a number of oblong, vitally stained cells. Around them there is a rather broad cleft, which is clearly separated from the nervous parenchym by the stained limit. gliae. On this membrane a few vitally stained cells are found. (intima piae).

The ependyma cells are not coloured.

Stained ganglion cells, outside of the meningitic region and at some distance of the haemorrhage, cannot be indicated.

b) Description of the microscopical aspect of the mesencephalon:

The pia is here and there filled up with large round cells. Distinctly blue are the pia septa, and the vessels which accompany them. The ganglion cells are colourless.

The ependyma of the aquaduct is unstained.

c) Medulla oblongata and cervical region: like the mesencephalon.

Analogous facts may be communicated about Rabbit CC: Microcopic investigation of the telencephalon again shows considerable swelling of the pia and accumulation of pyrrhol cells (vitally stained).

The ganglion cells are colourless.

The spinal cord of Rabbits G. and CC. is free from dye. In the pia, however, vitally stained cells are found.

The plexus, too, shows light vital staining of its epithelia.

Consequently after endocranial, intra-arachnoidal application of trypan blue, vital staining occurs in the pia and its cells, in the perivascular cells and the membrana elastica interna.

The nervous tissue itself remains colourless.

If the injection is made less carefully, or if lesions are made in the nervous tissue itself, intraparenchymatic haemorrhages arise, followed by necrosis of nervous tissue and staining of a few dead ganglion cells. Probably the dye has then come directly into this region and has been taken up by the dead tissue.

Those coloured parts are always very small, which pleads against a diffusion of the dye. That the death of the cells has not been caused by trypan blue itself, may be proved by the fact that the animals remain clinically intact.

In the following pages I shall try to prove that the toxicological symptoms, as described by Goldmann, are caused by a direct injection of the dye into the nervous tissue itself.

Fourth series of Experiments.

Injection of trypan blueinto the nervous tissue itself.

The problem to be studied with this series was how and how far trypan blue (as a prototype of the acid benzidin dyes) penetrates into the nervous tissue after intracerebral injection.

I also studied in detail how an animal reacts upon the intracerebral application of drugs, especially in connection with the experiments of Bruno. (Chapter III).

I made my experiments under different conditions:

The quantity of liquid injected was varied; the animals were allowed to live for various lengths of time; the pressure of the injection was changed, whilst also the depth of the puncture into the brain tissue was taken differently.

Protocols:

No. I. Rabbit K.

20-6-'19: Ether narcosis. Section of the skin and periost.

The hemisphere, medial to the left wall of the orbit, is exposed. The pia in intact. The needle is put deeply into the nervous tissue and I c.c. of trypan blue is injected under rather high pressure.

During the injection the animal screams violently, although the narcosis is deep. The wound is closed in the usual way.

After awakening from the narcosis the animal is quiet for 10 min. Then violent opisthotonus and tonic contractions in the hind legs occur. They last for some seconds. Then the animal is quiet again, a period of rest which lasts for about 2 minutes.

Now the aspect changes: the animal runs quickly forwards, falls on one side, and shows tonic and clonic contractions of all its muscles.

While the rabbit lies on its side, its legs make quick movements. Within twenty minutes three similar attacks appear. The attack itself lasts about one minute. Between the attacks the animal is somnolent and very rigid.

Half an hour after the operation the animal is put into a warm kennel.

It remains very rigid, but is fairly quiet.

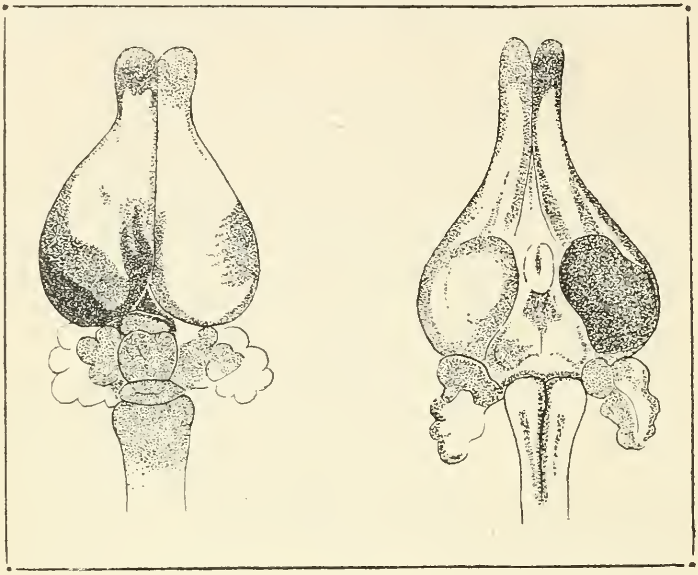
Then again a series of epileptiform attacks appears. About 5 hours after the operation the animal succumbs.

The body is still rigid, the head in opistotonus and the legs turned towards each other.

Autopsy: blue staining of the pia of cord and medulla oblongata. Intense staining of the hind part of the left hemisphere and of the rhinencephalon. The cerebellum shows a staining, which is less clear: the flocculi are colourless.

The convexity of the right hemisphere shows a light blue colour.

Fig. 6.



Rabbit K.

Intracerebral injection of Trypan blue into the left hemisphere. The dotted parts are stained blue.

Basis cerebri: its aspect differs much from the usual type of staining. Slight staining of medulla oblongata and pons. The basal part of the left rhinencephalon, however, is deep blue. as well as the other parts of the left hemisphere.

The right part of the basis cerebri shows hardly any blue colour. (fig. 6).

Description of the spread of the dye (studied in frontal sections). (fig. 7).

1) (III) To the right only the pia surrounding the rhinencephalon shows a blue staining, in contrast with the left part of the section. The basis of the rhinencephalon (till about the half of its height) is dark blue. The ventricle is filled with a blue substance.

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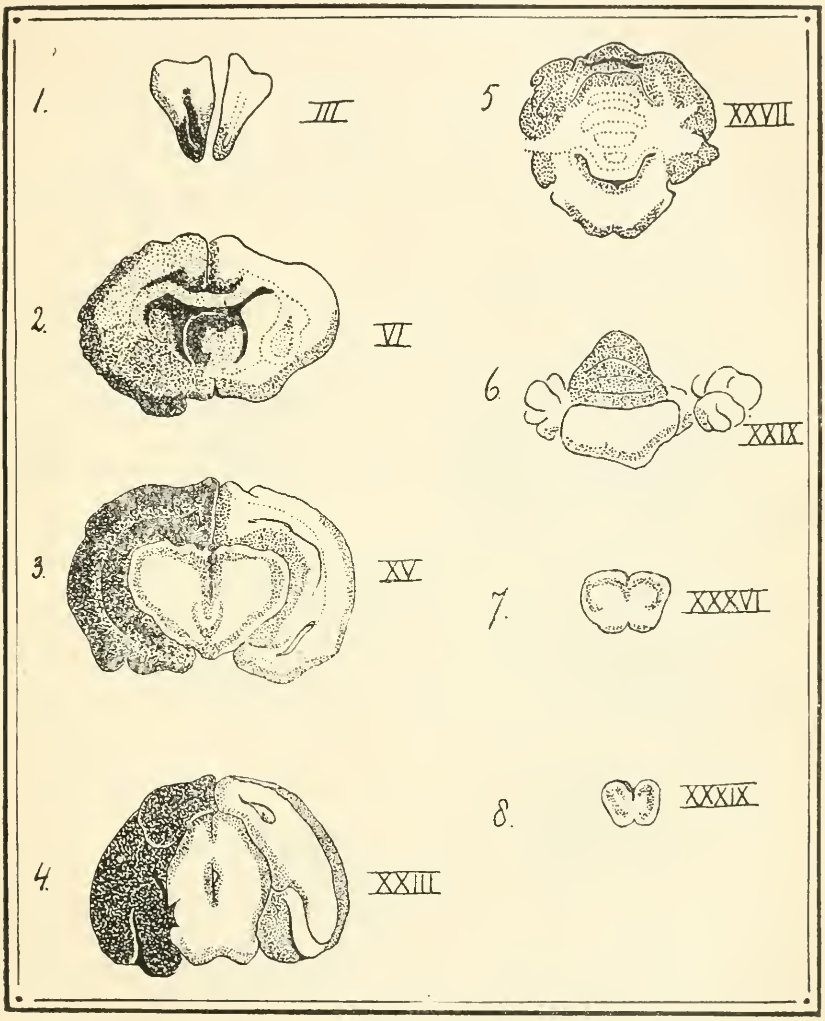
[1] The roman ciphers agree with those of the plates in "An anatomical Guide to experimental research on rabbits brain"(WINKLER and POTTER).

2) (VI-VIII). Only the left part of this section shows a blue staining. To the right only the ventricles and the pia are stained. The aspect of the left part of the section is a more complicated one: the ventricle is distinctly blue, as well as the choroid plexus, which is visible in it.

Fig. 7.



Rabbit K.

Intracerebral injection of Trypan blue. Frontal Sections.
(The roman ciphers indicate the plates of the atlas of WINKLER and POTTER).

The lesions, caused by the puncture, are clearly visible: one at the level of the fissura rhinica, two in the left nucleus caudatus. Near these lesions the staining is intense, so that consequently rhinencephalon and nucleus caudatus, putamen and fornix are dark blue.

Also the periphery of the whole telencephalon is stained distinctly.

3) (XV) The right part of this section shows an analogous aspect to those described with the endolumbal injections.

Staining of the pia, which surrounds the telencephalon and diencephalon, and of the right lateral ventricle.

The left part of the section is distinctly blue: a lesion is to be seen about 4 m.M. above the fissura rhinica, passing into the ventricle.

The most intense staining is visible in the nervous tissue surrounding the ventricle, and at the periphery of the telencephalon.

4) (XXIII) Light blue staining of the pia surrounding the right hemisphere (in the section the area occipitalis, rhinica and retrospleniale are visible).

The mesencephalon is cut through the distal part of the corp. quadrigemina ant.

The aquaduct and the surrounding pia are light blue.

The distal part of the left hemisphere is clear blue, just as its surrounding pia (diffuse staining).

5) (XXVII) Blue staining of the intercerebellar septa, and of the pia surrounding the pons. Diffuse staining of the ependyma lining the fourth ventricle.

6) (XXIX) Just as fig. 5.

7) etc. Sections through medulla oblongata, etc.: light staining of the surrounding pia.

Consequently I may conclude from this protocol that intracerebral injection of trypan blue causes a number of symptoms, which may be compared with Goldmann's results after intraspinal injection.

The dye has spread in the region of the injection, to the ventricles and to the cerebrospinal fluid.

No. 2. Rabbit M:

11-7-'19. Ether narcosis. The left hemisphere is exposed and 1 c.c. of trypan blue injected into it. (Several punctures are made, all near the sagittal line).

After awakening from the narcosis, the rabbit is dull and lies on one side. This lasts for about a quarter of an hour.

Then it suddenly moves quickly forward and shows clonical contractions of jaws and extremities. This lasts for 2 minutes. After this the animal falls on one side, with the legs turned towards each other. The attacks (clonical contractions, etc.) are repeated every ten minutes.

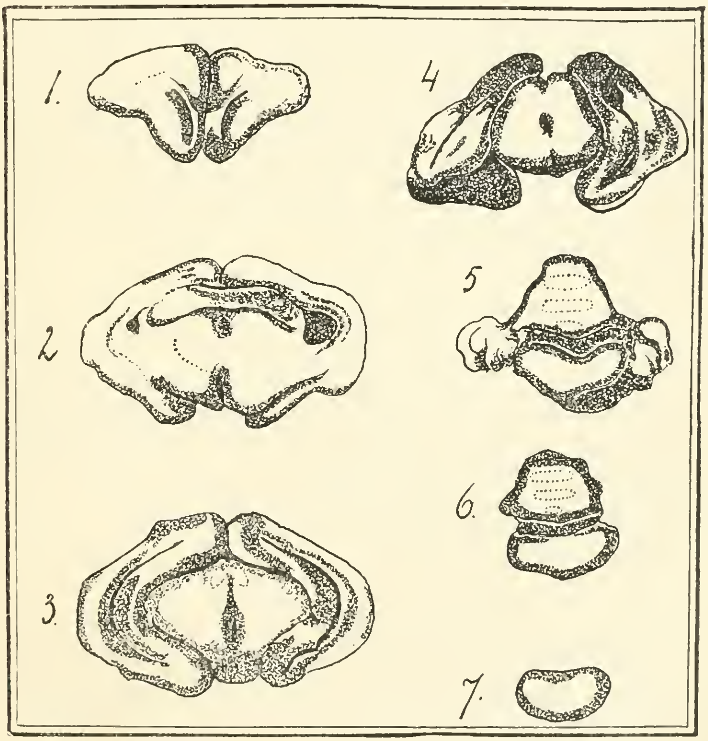
Death about 5 hours after the operation.

Autopsy:

pia of the cord — dark blue.
,, ,, ,, med. obl. — dark blue.
,, ,, ,, cerebell. — dark blue.
both hemispheres — dark blue (especially their medial parts)
basis cerebri — dark blue.

Description of frontal sections through the nervous system. (microscopical aspect) (fig. 8):

Fig. 8.



Rabbit M.

Intracerebral injection of Trypan blue; the dotted parts are dark blue. (Frontal Sections).

The ventricles and the aquaduct show an intense staining, as does a zone of nervous tissue surrounding them (section XX).

The section, corresponding to the plate XX of the atlas of Winkler and Potter, and showing the mesencephalon in the region of ganglion interpedunculare and nucleus oculomotorius, shows a deep blue staining of the aquaduct and the surrounding nervous tissue. (4).

Also a zone of tissue of 1 m.M. at the periphery of the mesencephalon is stained deep blue as well as the surrounding pia.

The tissue at the medial side of the hemispheres is stained to a depth of about $1^1/_2$ m.M.

This description holds good for all other sections. The microscopical description will be given afterwards.

No. 3. Death a long time after the injection.

Rabbit D. 13-6-'19. The left hemisphere is exposed at the medial side of the wall of the left orbit. The pia is intact. The surface of the brain is injured by a scalpel and 1 c.c. of trypan blue is injected into the brain substance at different places.

Strong dilatation of the pupils. The animal is restless. Adduction of the fore limbs and stretching of the hind limbs. If the animal is irritated by stamping on the ground, it jumps forward, falls on one side and shows a strong opistotonus. From time to time the animal runs round in a circle. This lasts for ten minutes. Then the animal becomes quieter and sits down in its straw and seems apathetic.

14-6-'19. The animal moves well. Does not eat however. No change in the gait.

Death by bleeding.

Autopsy:

Distinct staining of the pia of the cord and of the convexity of the hemisphere. The cerebellum is light blue, the flocculus excepted. At the basis cerebri, the medulla oblongata only shows a staining in its medial parts. The pons, pes pedunculi and occipital lobes are light blue.

The sheath of the N. opticus is dark blue.

A lesion exists in the anterior part of the rhinencephalon.

Microscopical examination of frontal sections: light blue staining of the pars anterior rhinencephali, of the ependyma lining the ventricles, and of zones surrounding the ventricles.

Light staining of the pia.

What may be concluded from the description of the nervous systems of rabbits M. and D.?

1) that, by intracerebral injection under high pressure, it is possible to cause symptoms which entirely agree with those observed by Goldmann after intraspinal injection.

2) that the dye has spread through the nervous system in a typical way, viz. staining of the tissue near the injection, whilst the rest is localized round the ventricles and at the periphery of the nervous system (where it is in contact with the cerebrospinal fluid).

An explanation of these facts will be given afterwards.

Intracerebral injections may be made, without raising serious symptoms. This results from the following experiment:

No. 4. Rabbit Q.

2-8-'19. Injection into the left hemisphere of $^1/_2$ c.c. of trypan blue under a low pressure.

Two minutes after the injection clonical spasms of the whole body occur.

The animal screams after awakening from the narcosis. It falls on one side (after about 15 minutes).

The animal recovers completely; takes food, is lively and does not show any abnormalities.

I may in my opinion, conclude from the protocols mentioned above, that the clonical and tonical spasms, etc. result from a direct lesion of the nervous tissue by the intracerebral injection of trypan blue.

That other fluids may have a similar influence, results from the following protocol.

No. 5. Rabbit Y:

28-9-'19. The left hemisphere is exposed. Intra-arachnoidal injection of 1 c.c. of a 4% solution of potassium iodide.

No symptoms arise.

10-10-'19. Again the left hemisphere is exposed. Intracerebral injection of $^1/_2$ c.c. of the solution of potassium iodide.

Three minutes after the injection the animal shows clonical spasms of the hind legs, the jaws, etc. After some time all muscles show clonical contractions.

The animal succumbs two hours after the operation.

From these facts we conclude as follows:

As after intracerebral injection of liquids, symptoms arise, such as spasms, etc., it may be accepted, with reserve, that Goldmann's results (with intraspinal injections) have an analogous cause, viz. injection into the nervous tissue itself or, at least, lesion of the nervous tissue by the operation.

Dewey says about these facts:

"I am inclined to believe that the symptoms do not arise so much from any toxicity of the staining substance, as that they are due to mechanical and physical factors and direct injuries of the nervous tissue in the process of the injections, which are not so easily controlled in the rabbit".

She saw serious symptoms occur after an intraspinal injection of aqua dest. or normal saline solution.

The following experience may prove this hypothesis:

No. 6. Rabbit CC.

31-10-'19. Ether narcosis. Exposure of the under thoracic region and intra-arachnoidal injection of 1 c.c. trypan blue solution.

Immediately after the injection typical symptoms arise: violent screaming and clonical spasms.

After some minutes the animal recovers completely.

After awakening from the narcosis, a paraplegia of the hind legs becomes visible; it passes quickly.

1-11-'19. Death by bleeding.

Autopsy: the nervous system is totally colourless.

INTRACEREBRAL INJECTION.

RABBIT	SPREAD OF THE DYE	SYMPTOMS OF INTOXICATION	PLACE OF INJECTION	DEATH
D	ependyma nervous tissue, surrounding the ventricles. Tissue, surrounding the place of injection	Typical symptoms Lasts for $^1/_2$ an hour	left hemisphere Not very deep.	Is killed after 24 hours.
E	idem	Typical symptoms. Last for an hour	left hemisphere deep.	idem
K	Intense staining of the tissue, surrounding the place of injection. Light blue staining of the ependyma, pia and surrounding zones of tissue	Typical symptoms. Last until death	left hemisphere very deep.	succumbs 7 hours after the injection.
M	same D.	idem	into both hemispheres (near the median line).	succumbs 5 hours after the injection.
P	idem	idem	left hemisphere deep	succumbs 3 days after injection.
Q	tissue, surrounding the place of injection	Typical symptoms. Last for 15 minutes.	left hemisphere not deep	lives for weeks, without showing neurological changes.

Microscopical investigation of the nervous system of Rabbit M:

In a section through the cornu anterior of the right lateral ventricle, the ventricle proves to be lined by a blue border, in which here and there dark blue spots are to be seen.

Also the pia is stained distinctly and is accompanied by a blue zone, in which equally dark blue spots are visible.

Higher magnifying explains those images: The ependyma of the ventricle proves to be distinctly stained. The staining is diffuse; protoplasm and nucleus are dark blue.

Most ependyma cells show blue offshoots, which penetrate into the nervous parenchym. The ependymal lining, however, is not a continuous one: in some parts of the ventricle no ependyma at all is to be seen and the nervous tissue lies bare.

The nervous tissue surrounding the ventricle is stained blue. The fibres show a light blue staining, the ganglion cells, however, are stained more intensely (diffuse staining).

According to the distance from the ventricle, the intensity of staining decreases, till we see a territory (between ventricle and surface of the brain), where no stained cells occur. In the pia oblong cells occur, which are diffusely stained, whilst in a zone at the periphery of the telencephalon the same distribution of the dye may be observed as in the zone surrounding the ventricle: diffuse staining of ganglion cells.

The intensity of this staining runs parallel with that of the pia.

Consequently we have observed in this (and analogous) sections: a diffuse (not vital) staining of the ependyma lining the lateral ventricles. Surrounding the ventricle we see a light blue zone, in which many diffusely stained ganglion cells are to be seen.

This zone is followed by one in which the cells are not stained, whilst under the pia, and lining the surface of the telencephalon, another zone may be observed, which shows a blue colouring (and many diffusely stained cells).

The same may be said of sections through the mesencephalon, medulla oblongata and cord.

The ganglion cells, in the neighbourhood of the central canal, are diffusely stained; the cells of the cornu anterior have not fixed the dye.

The pia is diffusely blue; together with a zone of nervous tissue surrounding the section of the cord.

Microscopical examination of the nervous system of rabbit P. gives corresponding results: diffuse blue staining of ependyma and pia, and of two zones of nervous tissue accompanying ventricle and surface.

(The following facts have been noted on the behaviour of rabbit P.: intracerebral injection of $^1/_2$ c.c. trypan blue. Spasms, convulsions, rigidity of the animal).

What interpretation must be given of the results obtained?

We inject into the brain substance, under high pressure, a large quantity of fluid. That the injected quantity is large in relation to the quantity of brain tissue, may result from the fact, that the weight of a rabbit's brain is light.

We not only inject trypan blue into the brain tissue, also the ventricles and the arachnoid cavity receive a part of it.

Intracerebral injection of different other substances produces analogous symptoms, as those caused by trypan blue .

Consequently we may not ascribe them to a specific action of trypan blue.

Another result of the intracerebral injection is a diffuse, non-vital staining of the tissue in the neighbourhood of the place of injection, together with an analogous colouring of the ventricular ependyma, the pia, and zones of brain tissue surrounding ventricles and pia.

As may result from the description of the intraspinal injection, a diffuse staining (non-vital) of the elements and pia only occurs if preceded by a lesion of those elements.

Thus, at first death of the cell, followed afterwards by non vital staining. The spread of the non-vital staining in rabbits, which are injected intracerebrally, makes plausible to me the following consideration:

Since I inject under a high pressure a liquid into the nervous parenchym, the ventricles and the cerebrospinal fluid, the relations in the pia, ventricular ependyma and accompanying nervous tissue are changed in such a way, that the cells of those organs are altered and even destroyed. The dye, being capable of staining all dead protoplasm, will stain those dead cells in a diffuse way.

Probably the symptoms which occur after the intracerebral application of other substances may be interpreted in an analogous way: death of the tissue in the neighbourhood of the place of injection, of the pia, ventricular ependyma and zones of nervous tissue accompanying the ventricles and pia.

The symptoms, arising after intracerebral injection of all sorts of substances, agree in such a striking way, that the explanation mentioned may not be considered as an irrational one.

For though I inject into one hemisphere, bilateral rigidity occurs. I may conclude from these experiments that substances with a low rate of diffusion, injected intracerebrally, do not penetrate deeply into the nervous system.

For the relation of these experiments to the therapy of lues nervosa, I may refer to chapter VII.

Fifth series of Experiments.

The experiments of Goldmann had shown that only after intraspinal application of drugs a pharmacological action on the central nervous system may be expected. The intravenous application proved to be inactive: no staining of the nervous tissue occurred.

According to him, this may be ascribed to the protecting function of the plexus.

In connection with the fact that (according to most neurologists) analogies exist between vital dyes and salvarsan, the intraspinal salvarsan treatment of lues nervosa became a routine method.

Each author on intraspinal treatment quoted these experiments, and considered the plexus as the protecting membrane of the nervous tissue, not only against poisons, but also against important drugs.

Recently Gennerich wrote in this respect:

(p. 10). „Ob es überhaupt möglich sein wird, ein Salvarsanpraeparat zu konstruieren das zufolge eines besonder günstigen Permeabilitätskoeffiziënten für die Plexus Choroidei ein gleich intensive Wirkung auf Liquor und Meningen, wie auf die gut durchblutete Organe besitzt, läszt sich zur Zeit noch nicht übersehen".

I have, though not expecting any succes, studied the question, if we may consider the non-penetration of trypan blue after intravenous application, as a result of the function of the choroid plexus.

Extirpation of the plexus was the best way to follow now:

the isolation of the whole plexus however was an impossibility, so that I was compelled to find another method of attaining the plexusless state.

As an example the protocol of Rabbit Z. may be mentioned:

The animal was etherized. Removal of the ossa parietalia, etc. Bleeding from the sinus in the left cerebellar edge, which is immediately stopped.

In small parts first the left, afterwards the right hemisphere are removed with the object of removing at the same time the principal parts of he plexus.

The respiration, etc. remain unaffected. The metencephalon is not cut away: it moves regularly by the heart beat.

Slight bleedings are stopped.

At the end of the operation some liquid paraffine is poured on the basis cranii, in order to stop eventual bleeding. The wound is closed in the usual way.

The animal is totally passive; the respiration is slow, the pupils are wide, cornea reflexes positive.

No active movements at all.

Gradually (after about 15 minutes) the respiration begins to grow regular. The temperature of the animal increases.

After 30 minutes 10 c.c. of $^1/_2$% solution of trypan blue are injected into the right marginal ear vein.

2 u. 30 p.m.: The animal is passive, respiration is slow.

The conjunctivae bulbi are stained blue. Bleeding in the wound.

8 u. 30 pm.: The animal lies with its legs stretched straight forward.

Great rigidity of the hind legs. Dorsal flexion of the head. The respiration is frequent, but regular (80 pro minute).

From time to time the animal utters a plaintive sound.

Bij lifting up the skin of the back, defensive movements of the fore legs are elicited. The rabbit tries to stand on the hind legs, but falls down again.

While lying, the limbs make walking-movements.

The conjunctivae are intensely blue.

The next day: The rabbit has died.

Autopsy: Skin and subcutaneous tissue are blue.

The fat is colourless. Deep staining of organs, of dura and of the intervertebral discs.

The nervous tissue is colourless.

Large coagulum clots are to be seen in the cavity of the skull. The whole animal is fixed in formol 10 %.

Of the brain the following parts are intact:
the cerebellum.

a part of the metencephalon.

Between cerebellum and pons a large coagulum clot is visible. The plexus of the 3d ventricle is surrounded by it.

Microscopical examination of the nervous tissue: The nervous tissue is colourless.

In the pia here and there vitally stained cells are to be seen.

In the internal organs the common staining has appeared: e.g. staining of Kupffer's cells in the liver, connective tissue cells, etc.

I may conclude from this experiment that, notwithstanding the plexus has been removed and the vascular supply of the remaining tissue is sufficient, no trypan blue may be found in the nervous tissue.

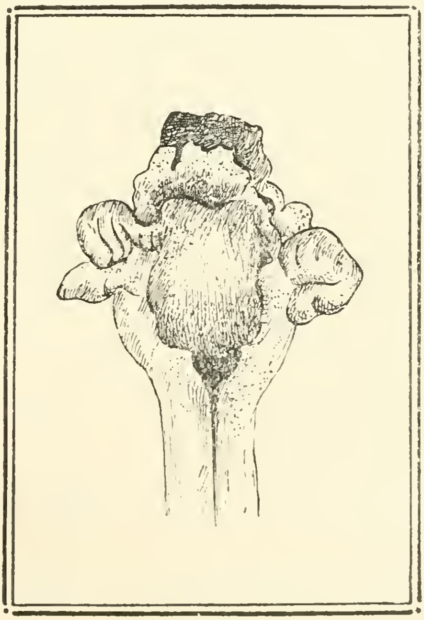
On the contrary, vitally stained cells occur in dura, pia, internal organs, etc.

Consequently, removal of the plexus does not create the possibility of fixing trypan blue to the nervous tissue.

Rabbit I. (Died $1^1/_2$ hour after the operation).

10. The animal is etherized. The brain is exposed by removal of the parietalia, etc. Bleeding from one of the deep veins of the skull occurs. It is stopped.

Fig. 9.



Rabbit I.

Removal of fore brain and a part of the cerebellum, in order to extirpate the plexus.

In the beginning I try to remove the plexus from the ventriculus lateralis after opening the ventricle. The basal ganglia are visible, whilst the plexus cannot be found. Consequently the fore brain is totally removed (by means of a sharp spoon), as well as the corpor. quadrig. posteriores.

The cerebellum is exposed: removal of the cerebellar hemispheres and the flocculus. I do not succeed in completely removing the plexus of the IVth ventricle; a part of it, however, can be removed.

The respiration is irregular.

11.15. Intravenous injection of 8c.c. $^{1}/_{2}$ % solution of trypan blue.

Subcutaneous injection of 3 c.c. $^{1}/_{2}$ % solution of trypan blue.

The respiration is very irregular.

11.35. The animal dies suddenly.

The onjunctivae are blue.

Autopsy: (fig. 9)

Nervous system is colourless. Light blue staining of the pia. Internal organs: light blue.

Fixation of the animal in a 10% solution of formaldehyd.

Microscopical investigation (frozen sections): The same results as with rabbit Z:

The nervous tissue is colourless.

In the pia vitally stained pyrrol cells are visible.

SUMMARY OF THOSE EXPERIMENTS.

RABBIT	STAINING	SYMPTOMS	REMOVED PART OF THE BRAIN	DEATH
W	organs: blue pia : „	coma, lasting for 10 minutes. rolling movements.	The fore brain is removed, up to the chiasma.	after 24 hours
Z	idem	coma. Decerebrated rigidity	removal of the brain up to the cerebellum	idem
A A	idem	coma. Decerebrated rigidity	idem	after 12 hours.
I	organs: blue pia: very light blue	coma	idem + parts of the cerebellum	after 1. h 30 min.

Sixth series of Experiments.

Though qantities of trypan blue have been injected, sufficient to raise a distinct staining of the organs, we never, notwithstanding a nearly total extirpation of the plexus, see a blue staining of the nervous tissue.

We have made in the fifth series of experiments, the nervous system to an unprotected organ in the sense of Goldmann.

But, though we may observe after intravenous injection, a staining of spleen, liver, etc., in the nervous system no dye is to be found.

Consequently we must not consider the plexus merely as a protecting membrane of the nervous system.

In the vessels of the nervous sytem, trypan blue circulates.

The plexus, nearly totally removed, is not capable of storing the dye any longer.

Thus the dye remains in circulation in the vessels of the nervous sytem, and is always in contact with this organ, but we never observe staining either of nerve cells or of fibres.

The pia is always stained light blue: this results from the presence of vitally stained pyrrhol cells.

This raises doubt as to the conception of Goldmann and others: May we even consider the choroid plexus as the protecting organ of the central nervous system?

Do all the substances, which penetrate into the cerebrospinal fluid or stain the pyrrhol cells, pass through the plexus?

If we consider the plexus as the protecting membrane of the nervous system, this would be a matter of course: we may accept then that the poisonous substances reach the nervous system via the vessels of the plexus, but are removed selectively by its cells.

The question arises whether, via the other vessels, also dyes or poisons penetrate into the nervous tissue.

We may answer this question by making a part of the nervous system independent of the influence of the plexus.

I have done this in the following way:

Rabbit O:

The rabbit is etherized. The upper part of the thoracic region is laid bare to a distance of 4 c.M. A piece of cord is removed (at 1 c.M. of length). The space formed is filled with paraffin. Consequently I made a barrier between the oral and caudal part of the arachnoid cavity.

The wound is closed as usual. Immediately after the operation an intravenous injection of $7^1/_2$ c.c. of the trypan blue solution is made.

The animal is quiet: paraplegia of the hind part of the body.

It succumbs nearly 15 minutes after the injection.

Autopsy: the organs are light blue. Nervous tissue is colourless.

The above protocol has no value as a proof, but may serve to give an idea of the followed method: I have made two nervous systems, each with its own arachnoid cavity.

From the oral-cavity no fluid can flow towards the caudal nervous system, so that the latter part has its own vascular system.

Rabbit S.

10 a.m. The upper part of the thoracic cord is exposed. Removal of a piece of cord, of 1 c.M. length. Between the caudal and oral part of the cord a piece of fungus is applied on which paraffin is poured. The wound is closed in the usual way. Intravenous injection of 5 c.M. of a trypan blue solution. Paraplegia of the hind legs. — The rabbit is quiet.

8 u. pm. The animal is rather well. Takes food as usual.

Intravenous injection of 2 c.c. of trypan blue. Subcutaneous injection of 10 c.c. of trypan blue.

The next day: intravenous injection of 3 c.c. of trypan blue. The animal seems to be well.

Two days later: the state of health is bad: the rabbit neither moves nor takes any food.

The paraplegia is as two days previously. The hind part of the body is analgetic.

Blue conjunctivae.

OPHTALMOSCOPIC INVESTIGATION: The fundus oculi is not blue.

8 u. pm.: the animal is killed by bleeding.

Autopsy: The nervous tissue is colourless.

The pia is light blue, the upper part of it, as well as the part under the transverse section.

The organs show the typical staining. The plexus is light blue.

Microscopical examination:

The nervous tissue has not stored any dye. In the pia vitally stained pyrrhol cells occur.

(These results agree entirely with those observed in Rabbits S and O).

It may be concluded from the above description that, though the under part of the cord is excluded from the influence of the plexus, still a blue staining of the pyrrhol cells in this part may be seen.

Consequently we may conclude that trypan blue can penetrate into the nervous system, by following other ways than the vessels of the plexus.

Seventh series of Eexperiments.

Intending to complete my investigations, I tried to follow another method of making the plexus permeable for different substances.

I did not expect much from those experiments; still it was necessary to follow this method, especially in connection with the modern therapy of general paresis.

Wagner von Jauregg, and others, had treated their paretics by means of a combined treatment: fever and salvarsan.

And as it is known that general paresis often occurs in protators (after a syphilitic infection), the question arose as to whether in these cases the permeability of the plexus and other protecting membranes, had increased, as caused by the action of alcohol.

I made use of amyl alcohol, especially since the disagreeable results of gin, etc. must be ascribed to the higher alcohols.

Another factor of importance was that those alcohols decrease the surface tension: and as it is known that by lowering the surface tension, the diffusibility of substances into other materials increases, I tried to make use of this fact.

The permeability of the choroid plexus (or protecting membranes) should be studied equally under other conditions, such as infection, refrigeration and exhaustion.

The last condition was difficult to realize: the rabbit is more inclined to jump than to walk.

In a turning wheel-box it walked for a few moments, but soon the animal became totally passive, so that no exhaustion could be provoked.

Infectious processes equally were difficult to provoke.

It may, however, be mentioned that u blue staining of the nervous tissue was never be seen after intravenous application of trypan blue. The rabbits always remained neurologically intact.

As an example, the protocol of RABBIT KL. may be commucated:

Since the end of 1919, the animal has been injected subcutaneously every day with 2 c.c. of aethyl alcohol.

Afterwards injections with iso-amyl alcohol are made.

The animal has also been injected intravenously with solutions of trypan blue.

23-3-'20. $22^1/_2$ c.c. trypan blue are injected intravenously.

9-4-'20. The rabbit emaciates. Dyspnoea. Takes little food. Quick respiration. 15 c.c. of trypan blue are injected intravenously.

16-4-'20. The animal succumbs.

Autopsy: Light blue staining of the pia. Dura matris and intravertebral discs are distinctly blue.

The nervous tissue is colourless.

Lungs: have changed into a bluish, liquid mass.

Liver: dark blue.

Bowels: light blue.

Kidneys: blue.

Mucous membr. of the Bladder: blue.

Conjunctivae: blue.

Heart: light blue.

From the facts mentioned above I may conclude that the storage of trypan blue in the nervous system cannot be provoked by different exogenous factors.

CHAPTER V.

Discussion of my results, in connection with the theory of vital staining.

Goldmann found that, after intravenous application of trypan blue, the nervous tissue remained colourless. Other organs appeared to be stained intensely, but of the nervous system the choroid plexus was the only part stained.

He tried to account for this by supposing the choroid plexus to be a barrier against the entry into the cerebrospinal fluid in such cases.

If the dye was injected intraspinally, the nervous system was stained diffusely. Then the choroid plexus could not function as a barrier and the poison would act directly upon the nervous tissue.

My own results lead to other conclusions.

The results of the intravenous injection are quite in accordance of Goldmann. Only definite groups of cells appeared to be stained, especially cells with a specific character, e.g. cells of Kupffer, the „fixe Bindegewebszellen", the cells of choroid plexus, etc.

The results obtained with the intraspinal and endocranial application of the dye differed totally from those of Goldmann. The nervous tissue only proved to be stained after lesion of its elements.

After intraspinal application of trypan blue the nerve cells of the spinal cord proved to be colourless; only that part of the cord which corresponds to the place of the injection appeared to be stained.

Microscopical examination of this part showed that the tissue was altered intensely (necrotic spots, hemorrhages, etc.) Examination of other parts of the cord showed a different spread of the dye; the nervous elements were colourless, only the pia, septae and the rest of the connective tissue appeared to be distinctly stained.

The staining of most pyrrol-cells was a vital one (the nucleus being unstained.) Only pyrrol-cells in the neighbourhood of the punctured place were diffusely stained.

Endocranial injection gave analogous results.

So the hypothesis of Goldman must be discarded: neither after direct nor after indirect (intravenous) application of trypan blue on the nervous system, does a staining of nervous elements result.

Consequently the plexus may not be considered as a protecting membrane of the nervous system (at least for trypan blue).

In this relation the following questions arise:

1) The nervous elements, can they be coloured by acid vital dyes?

2) What is the reason why other groups of cells (pyrrol-cells) show a tendency to store vital dyes?

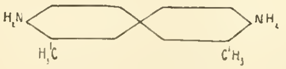
In the following pages I will endeavour to give a description of the chemical properties of the acid dyes, and to answer the questions mentioned above.

A. The chemical properties of acid dyes.

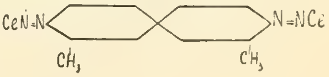
It has been proved from the investigations of Ehrlich that especially the basic dyes and their salts are capable of staining vitally animal cells.

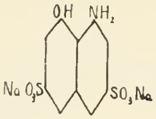
After the demonstration of the fact, that some acid dyes were likewise vital stains, these also drew the attention of many investigators. Especially trypan blue, the prototype of those dyes, was studied. It is prepared by adding ortho-tolidin to amidonaphtol.

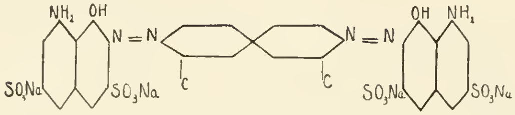
O-tolidin:



is diazotated to



The addition of two molecules of 1—8-amidonaphtol 3—6 disulpho acid,  gives us the following formula.



The solution of the dye is intensely blue and is not changed by ammonia and diluted acetic acid.

Addition of strong acids produces a blue precipitation, whilst strong alcali makes a violet one.

Serum, however, does not produce precipitation in solutions of trypan blue, in contradistinction with the other acid vital dyes, pyrrol and isamin blue.

Ultramicroscopically no particles can be made visible in the solution. The dye is little diffusible into gelatine.

Von Möllendorf, however, has shown by d~~y~~al~~i~~sis, that /i Ly trypan blue is composed of two dyes, a red substance which is highly diffusible and a blue one which is less diffusible. I could state this observation.

Schulemann especially studied the vital dyes as to their physiological and their physical properties.

He found that dyes which are little diffusible only stain vitally the place of injection.

The increase of the power of vital staining runs parallel with the rate of diffusion in gelatine. Dyes with an average rate of diffusion are the genuine vital dyes and very suitable for histological purposes.

If the rate of diffusion increases, the interval between injection and excretion of the dye decreases and the organism loses its colour in a short time.

If the dye is highly diffusible, it is not stored in definite cells but distributed over the whole organism.

According to Biltz, the acid dyes, especially those with high molecular weight, may be considered as semicolloids.

Their rate of diffusion is slow; at the same time they have an osmotic pressure. They evidently stand between colloids and crystalloids.

Those semicolloids incline to aggregation, so after some time their solutions acquire a colloidal character.

The physical properties of the vital dyes, mentioned above, give a general explanation of their storage in the animal body. Regarding their fixation in special groups of cells they do not give us sufficient information.

How the dyes are stored in those cells has been the subject of much discussion. Goldmann for one believes that the dye is fixed to preformed granules, whilst Schulemann assumes that the dye granules, which are visible in the cells, are formed by the acid dye itself.

He was able to demonstrate this by staining simultaneously with two dyes. So he injected in mice the first day 0.5 c.c. of a 1 % solution of trypan blue, and the following day the same mice were injected with as much vital neurot. The mice were killed the third day. It appeared that each stained cell was filled with red and blue granules.

If vital staining is the result of a tinction of preformed granules, then we might expect violet granules in the cells.

The objection may be made to this experiment that possibly, in the interval between the two injections, new granules were formed which afterwards fixed the vital neurot.

If, however, a mixture of trypan blue and vital neurot $\overline{aa}$ is injected, red and blue granules still appear.

From this Schulemann concludes that the microscopical aspect of vitally stained cells, is a result of the presence of granules, formed by the aggregation of dye particles.

He has given proofs of this hypothesis by following another way of investigation:

He saw in vital staining with acid dyes (e.g. congo-rubin), according as the injected dye was increased, metachromasy occur from red to blue.

That this is the result of the formation of conglomerates, could be demonstrated in reagent tube experiments. The addition of electrolytes to solutions of acid dyes, darkens their colour.

Schulemann concludes: „Die Speicherungsgranula in den Zellen entstehen erst unter dem Einflusz der sauren Farbstoffen". (p. 117).

He gives the following conception of the mechanism of vital staining:

Under the influence of the penetration of dye solutions into the protoplasm, vacuols are formed, in which storage of the dye occurs. If the quantity of dye in the protoplasm has attained a certain degree, the dye will agglomerate, and dye particles will be formed. These particles will increase in number and size, and finally dye granules (consisting of agglomerated dye particles) will become visible. So vital staining may be considered as a process of coagulation of colloidal solutions (dye solutions) under the influence of the addition of electrolytes (which are dissolved in the protoplasmatic fluids).

Other theories of vital staining are those of Ruhland and Overton.

Ruhland believes that the cell membrane is an ultra filter, so that the possibility of penetration of dye granules into the protoplasm depends totally on their size.

The objection may be made that, according to Ruhland, each cell can be stained vitally provided the grade of dispersion of the dye be a high one.

It is evident that this is not possible; only special groups of cells are capable of being stained vitally.

The lipoid theory of Overton likewise is not capable of giving an explanation of the process of vital staining. By many cells, „lipoid-unlösliche" dyes are taken up, whilst, on the contrary, some „lipoidlösliche" dyes are not stored at all (victoria blau, etc.).

Other objections to those theories (of Overton and Ruhland) might be mentioned in this paper. I refer, however, to the explanations of Höber, in his „Physikalische Chemie der Zelle und der Gewebe".

Summarizing the following conclusions may be made from the preceding pages:

Trypan blue is an acid dye, that stains definite groups of cells, but no nervous tissue.

It has the properties of the so-called semicolloids, inclines to aggregation and diffuses at a low rate.

The vital staining is not a staining of preformed granules, but must be ascribed to an accumulation of dye particles in the protoplasmatic fluids. (coagulation of semicolloids).

The theories of Ruhland and Overton do not explain why merely definite cells take up the vital dyes. They consider the cell as a passive organ; according to Ruhland it consists of protoplasm surrounded by a perforated membrane.

Overton considers this membrane as consisting of lipoids and also ascribes an important function to it.

The fact that the vital staining is a selective one makes it necessary to consider it as a biologic process.

In this connection Schulemann observed that the cells, which store the vital dyes, equally store other materials in an analogous way: e.g.

1) acid colloidal dyes
2) colloidal metals
3) bacilli
4) erythrocytes
5) chinese ink
6) bile pigments
)7 cholesterin (Anitschkow).

Apparently great differences exist between those substances. They agree, however, in the fact that they all may be considered as colloids or semicolloids and that in cataphoresis they move towards the positive pole.

Since the charge of all sorts of particles is highly influenced by the presence of crystalloids, the analogy between the substances mentioned above as to their charge, does not hold in all cases.

In the blood their charge probably will be a different one.

The size of these particles varies from 15 microns till ions.

Since it is certain that the smallest particles are taken up by the same cells as the larger ones, it is reasonable to indicate this process by the collective name of „phagocytosis".

This hypothesis is supported by the investigations of Hall Downey. He endeavoured to demonstrate that the phagocytic cells of the blood are capable of storing acid dyes, though Goldmann and others had not observed vital staining of those cells.

It was known, however, that, though very seldom, phagocytosis may occur by leucocytes in the blood itself.

Bull and Adami for instance had demonstrated that after injection of microbes into the blood, they are stored by endothelium cells and leucocytes, which accumulate in the diseased organs. It proved that the leucocytes of the general circulation only store the microbes, in such cases, where the endothelium cells were not able to do so.

On the other hand, it was known, that the sort of cells, which act in phagocytosis, depends on the conditions in which the experiment is made (Lattan-Larrier observed, that even eosinophyl leucocytes could act as phagocytes).

From these facts, Downey concluded:

1) that phagocytosis is a physiological process, a property of different groups of cells.

2) that other groups of cells, depending on the conditions into which they are brought, may act equally as phagocytes.

Consequently it must be possible to make the neutrophile leucocytes of the blood store vital dyes.

Downey injected the dye into the vena femoralis, after double ligature of this vessel. If after 24 hours a microscopical investigation was instituted, all leucocytes proved to contain blue granules.

So the leucocytes may store vital dyes, provided special conditions be realized (in casu a condition of time).

This pleads for the phagocytary theory of vital staining.

„Intra vitam „staining" with the azo dye pyrrol blue is a process of ingestion and storage, and not one of true staining of preformed structures."

Later investigations of Downey demonstrated in another and easier way, the phagocytic action of the leucocytes for azo dyes. If $^1/_2$—1 hour after injection of 20—50 c.M^3. of trypan blue in rabbits, the blood was examined, all the leucocytes appeared to be filled with blue granules.

Consequently he concludes:

„Phagocytosis of colloidal dyes by poly-morphonuclear leucocytes depends entirely on the availability of the dye rather than upon any inherent physiological difference with respect to phagocytosis between these cells and the mononuclear macrophages of the tissue." (p. 113) [1])

In connection with the phagocytory theory of vital staining I may refer to the experiments of Schlecht and Kiyono. They injected lithion carmine and succeeded in indicating this dye in the same cells in which Goldman had demonstrated the injected trypan blue.

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[1]) By this theory of vital staining, especially by the investigations of Downey, the proof of the dualism in formation of the lymphoid cells (Pappenheim) has become impossible. (lymphocytes of the blood and „histiogene Zellen").

The method is not a specific one and thus cannot be used for the purpose of distinguishing between cells of tissue origin and those which have come from the blood.

According to Kiyono this fact cannot be explained from chemical properties of the dyes. Probably physical processes must be considered as its cause (adsorption).

He finds the following cells stained vitally:

1) the epithelium cells of the „Hauptstücke" of the kidney.
2) the liver-cells (though little).
3) the epithelium cells of the choroid plexus.
4) the endothelium cells of the serous cavities.
5) other endothelium cells.

In the preceding lines I have communicated some facts which plead for the phagocytary theory of vital staining. I have demonstrated that only definite groups of cells (fibroblasts and reticular cells of lymph-nodules) store the dye granules, and have compared, according to Schulemann the process of dye storage with the other phagocytary processes (of bacilli, etc.).

The question arises whether there are facts in physical biochemistry, which may give a plausible explanation of general phagocytosis.

It is known that phagocytosis and amoeboid movements are two properties of the cell which stand in close relation.

According to Berthold the movement of liquid masses in media of other specific weight, depends on changes in the surface tension.

Probably the amoeboid movements of leucocytes, etc. may be explained by analogous influences, and as it is known that the electric current lowers the surface tension, the question arises whether electric currents may occur on the surface of those protoplasmatic elements. (Hirschfeld).

The amoebe (the prototype of the phagocytic cell) moves in the electric field towards the negative pole, the bacillus and other substances (which are enwrapped by the amoebe, etc.) move towards the positive pole.

The bacillus is charged negatively and at a definite spot of the amoebe lowers its surface tension. Consequently phagocytosis occurs.

This hypothesis is supported by the fact that H-ions (e.g. lactic acid) increase the phagocytic action of leucocytes.

If I endeavour to draw conclusions from the considerations mentioned above, I must begin by putting the question: What is the reason that, after intravenous injection of benzidin dyes, no dyes can be demonstrated in the nervous system and that they are stored in definite groups of cells of the connective tissue?

Other dyes do the same, just as colloidal metals, bacilli, etc. None of those materials, however, can be indicated in the nervous system. All the materials which are stored in the cells of the connective tissue, etc. have a common property, viz. the low rate of diffusion and strong tendency to aggregation.

Those facts may be considered from one point of view, by assuming an active function of the cell, phagocytosis, a function which is influenced by physical processes (H-ion concentration, etc.).

The fact that after intravenous injection trypan blue cannot be demonstrated in the nervous system, is not caused by the so-called impermeability of the plexus, but probably is a consequence of the fact that the elements of the nervous system are not capable of storing the dyes or have not the property of phagocytosis.

This is formulated in the following way by Schuleman and Evans:

„Die vitale Färbbarkeit einer Zelle ist also allein das Zeichen für einen gewissen physiologischen Character des Protoplasmas einer bestimmten Kategorie von Zellen." [1])

Trypan blue is not only stored by definite groups of living cells; also dead cells (dead protoplasm) are capable of fixing the acid benzidin dyes.

This is not a typical phenemenon; dead protoplasm fixes all sorts of dyes, as well after as without fixation with solutions which coagulate the albumen, as we know from histological technics.

Fischel gives of this fact the following explanation:

„Die Eiweiszkörper sind aüsserst leicht veränderliche, überaus reaktionsfähige Körper; desgleichen sind saure und basische Färbsalze unter geeignete Bedingungen (eventuel Zusatz von Säure oder Alkali) in auszerordentlichen Grade befähigt, mit andern Chemischen Körpern sich umzusetzen."

Especially the investigations of Mac Curdey and Evans have demonstrated clearly that dead cells fix the acid dyes. They consider the diffuse staining of nucleus and protoplasm as absolute signs of the death of the cell. They studied polyomyelitis by means of intravenous injections of vital dyes and observed a diffuse staining of the dead ganglion cells of the ventral horns.

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[1]) These considerations have no direct significance for highly diffusible basic dyes. Their distribution in the organism may be compared with that of anorganic salts, etc.

The injured cells had stored the dye in granules.

In the wound, which was made by the inoculation of the polyomyelitic virus, the dead gliacells also showed a diffuse staining (the „Körnchenzellen" were stained vitally).

Bij those experiments it is proved that trypan blue is allowed to penetrate into the nervous system.

For we observe diffuse staining of the cells of the ventral horns, whilst the living ganglion cells are colourless.

Perhaps it is possible to explain this in connection with the preceding considerations: trypan blue circulates in the vessels of the nervous system, but is not stored, since the ganglion cells (the living ones) are not capable of storing vital acid dyes. If, however, the protoplasm of the ganglion cells is killed by the polyomyelitic virus, the dyes are fixed.

The investigations of Rost and Grosz likewise plead for this hypothesis.

If we accept Goldmann' hypothesis ("the plexus is the protecting membrane of the nervous system"), it should be impossible to explain why in polyomyelitis the dead cells are stained.

B. The chemical properties, etc. of salvarsan.

After the considerations on vital dyes, I will endeavour in the following lines to study the salvarsan group.

The following questions arise:

a) May we compare the salvarsan preparations with the vital dyes?

b) May we apply the considerations on phagocytosis, etc. to the salvarsan group?

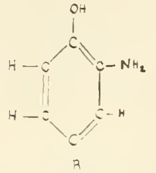
Those questions are studied in different ways, viz.

1) the chemical method
2) the physical ,,
3) the experimental and pharmacological method.

1) The chemical method.

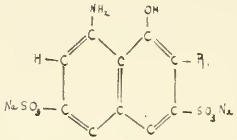
According to Ehrlich, the action of salvarsan is an evidence of a true chemical union between some part of its molecule (chemoceptor) and an element in the cell (chemoreceptor).

This chemoceptor, which occurs twice in the salvarsan molecule, has the following structure:



R is the toxophor group: -As = As- (in para-position).

Trypan blue has an analogous chemoceptor, though consisting of a naphtol-group:



R stands for the azo-group: -N = N-, which has a great analogy with the arseno-group.

On chemical considerations Kolle accepts a striking analogy between salvarsan and vital acid dyes.

According to him such a dye is characterized by:

1) a chromophoric group, which gives the dye the „physical property" of colour (-N = N-)

2) one or more auxochromic groups, which give the dye its property of staining.

„Im Salvarsan befindet sich die der Azogruppe ähnliche Arsenogruppe (-As = As), welche als chromophor wirkt, denn alle Arsenoverbindungen sind mehr oder weniger gelb. Ausserdem befinden sich im Salvarsan je zwei auxochrome Hydroxyl- und Aminogruppen.

Salvarsan hat also Farbeigenschaft und färbt demgemäsz auch Baumwolle, welche in üblicher weise mit Tannin und Brechweinstein vorgebeizt ist. Allerdings ist die Färbung lange nicht so stark, wie bei einem Azofarbstoff, sondern nur sehr mattgelb, woraus hervorgeht, dasz die Vereinigung von Chromophor und Auxochromen hier nicht so stark wirksam ist, wie bei den eigentlichen in der Textilindustrie angewendeten Farbstoffen."

Also Schuhmacher considers salvarsan as a genuine dye („echter Farbstoff"). According to him, salvarsan is not only a dye containing arsenic, but arsenic itself is a constituent of the chromophoric group.

From the facts mentioned above, we may conclude that, according to many authors, we may compare salvarsan with the azo-dyes.

2) The physical method.

The analogy vital dye-salvarsan must also be studied from the physical point of view, the stereochemical analogy in relation to their distribution being of less importance.

As has been mentioned above, the vital acid dyes may be considered as "semi-colloids", substances having a low rate of diffusion and a high inclination to aggregation.

It is made probable, according to Bauer, that the substances of the salvarsan-group have analogous properties.

The salvarsan preparations have a lower rate of diffusion than the crystalloids; it is, however, higher than the diffusion of genuine colloidal substances.

In the solutions of salvarsan no Tyndall-phenomenon is to be seen. Ultramicroscopically no particles can be made visible.

Bauer did not venture to conclude from those facts alone, that salvarsan forms colloidal solutions, and studied them by means of other methods. He made use of the ultra-filtration of Bechhold, a method by means of which colloids can be separated from crystalloids.

Salvarsan proved to filtrate totally.

An alkalized solution of salvarsan (as used generally in medical practice) proved, however, to be a mixture of small and large particles. If this solution was allowed to stand for two days, it proved that the unfiltrable residu had diminished.

Evidently peptisation had occurred.

Another curious fact is that the dissolved salvarsan dichlorhydrate cannot be filtrated.

Neosalvarsan, on the contrary, the undissociated natrium salt of salvarsan-formaldehyd-sulphoxyl acid, passes ultrafiltration without leaving any residu.

Sulphoxylate (No. 1495) on the other hand leaves a considerable residu. This pleads for its colloidal nature.

The diffusion-experiments of Bauer were performed in the following way:

He filled reagent tubes with a 3 % gelatine solution. After coagulation of the gelatin layer, the solutions that had to be examined (salvarsan) were poured into the tubes, which were closed hermetically. The tubes were allowed to remain for a fortnight in a refrigerator. At the end of this time, the solution was poured out, the tube was cleaned thoroughly, and heated for a moment in warm water, so that the column of gelatine could be loosened and taken out. The columns of gelatine were covered with a piece of blotting-paper, moistened with a solution of ammoniacal silver.

After some moments the salvarsan had blackened the papers to a certain degree, showing its rate of diffusion in the gelatine, which was photographed.

The experiments of Bauer, though very important in relation to our considerations on the action of salvarsan, do not stand the test of criticism.

The following objections may be made:

1) He does not mention precisely the physical and chemical properties of the gelatine he used.

Since it is known that the character of the gelatine, and especially its degree of acidity, has a great influence on the substances studied, it is necessary to avoid eventual misleading factors.

2) Bauer did not emphasize the influence of O_2 on the salvarsan-preparations. It is known that salvarsan is much changed by the oxygen of the air.

We may conclude from those facts that the experiments of Bauer must be considered with due caution, as long as no further details are given. 1)

Still it seems probable that salvarsan has a low rate of diffusion. Ultramicroscopically no particles could be made visible in solutions of salvarsan.

By the addition of small quantities of acid, particles can be made visible which disappear after addition of alkali.

It is known that acids have a coagulating action on colloids.

They produce an analogous action on a solution of salvarsan.

From this we may conclude that the salvarsan preparations are no common crystalloids, since we know that these substances are not coagulated by electrolytes. Probably we may consider them as standing between crystalloids and colloids (semi-colloids), since those semi-colloids (as mentioned above) have a low rate of diffusion.

According to the theoretical investigations of Sutherland, Einstein and Smoluchowski, the diffusion coefficient is inverse proportion to the radius of the particles.

From the experiments we may conclude that the particles of salvarsan are larger than those of crystalloids and smaller thans those of colloids, and that salvarsan and its derivatives (neosalvarsan, salvarsannatrium, silversalvarsannatrium) may be considered as semi-colloids, on ground of:

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1) Preliminary investigations made by myself, rendered those objections still more obvious.

In solutions of salvarsan precipitation occurred, even though every precaution was taken to exclude the air.

If this is merely a consequence of the presence of oxygen, cannot be ascertained merely yet.

1) the results of ultrafiltration.
2) „ „ of diffusion in gelatine.
3) the influence of acids on their dispersity.
4) „ „ of time „ „ „

It is not improbable that those semi-colloidal arsenocompounds act in the organism as colloids.

Conclusion from this part of the argumentation:

We know that trypan blue and other vital (acid) dyes are stored in definite groups of cells, resulting from:

1) a property of those cells, viz. the active process of phagocytosis,

2) and a definite property of those dyes (they are semi-colloids).

Neosalvarsan, the salvarsan preparation most used, moves, being a sulpho-compound, towards the positive pole. Salvarsan itself, however, has no sulpho groups in its molecule and probably in cataphoresis will not move towards the positive pole.

Schulemann tries to explain the distribution of substances, etc. in the organism, from their electric charge.

But since we know that the electric charge of particles in the organism is a variable one, it is possible that salvarsan (and other semi-colloidal substances) in the circulation change their charge.

I think it therefore allowed to change Schulemann's hypothesis on phagocytosis, by accepting an analogy in the distribution of semi colloidal and colloidal substances.

Salvarsan preparations may likewise be considered as semi-colloids, with the same physical properties as the substances mentioned above.

Consequently it is probable that salvarsan, just as the vital dyes, is stored in definite groups of cells with phagocytic properties. If by some cause anywhere an accumulation of phagocytic cells appears, it is probable that more vital dyes or salvarsan wille be stored than in other parts of the organism.

This raises the question, whether there may be facts which plead for this way of distribution.

3) On the distribution of salvarsan in the body as compared with that of vital dyes.

Goldmann studied in mice the tuberculosis of the peritoneum, and treated the animals with azo-dyes.

The peritoneum was covered with blue spots, which proved to consist of multitudes of „Netzzellen" (large mononuclear cells, in normal circumstances equally occurring in the peritoneum).

Those cells were stained vitally. They had stored besides the dye particles, large quantities of tubercle bacilli.

In the diseases of the lungs, equally vital staining of the infiltration cells may be observed; a rabbit, which had been treated for months with subcutaneous injections of amyl alcohol, sickened with a pulmonary process. At the same time injections of trypan blue were made into the marginal veins of the ear.

The autopsy proved that the lungs had changed into a liquid blue mass, consisting of blue cells (vitally stained).

Tschaschin made wounds in lymph-nodules, liver and spleen, and also observed, after intravenous injection of acid dyes, an intense vital staining of the infiltration cells.

The experiments of Clifford Macklin on brain repair in rats are also important in this regard.

The dead brain tissue was diffusely and deeply stained blue.

Vitally stained mononuclear cells appeared in the inflamed region at the end of the second day, and disappeared by the sixth day.

These macrophages occurring round the brain lesions were apparently recruited partly from the neuroglia, for hypertrophied neuroglia cells containing dye granules were found in the area of inflammation. Some also arise from the endothelium of the blood vessels.

A curious fact is that the quantity of dye taken up by the brain phagocytes is much smaller than that taken up by the mononuclear phagocytes in aseptic inflammation in other tissues.

The observations made on a small abscess, which occurred by accident in one of the cases, yielded other results. It consisted of an aggregation of pus cells, enclosed by a well-marked capsule of macrophages, which were stained dark blue. Outside of this macrophagic wall was a fibrous zone, which was directly continuous above with the pia-arachnoid. The abscess-wall macrophages were large, and more oval and elongated than those of the brain. From their close relation to the pia-arachnoid it follows that they may be developed from it and not from the glia.

We may conclude from the investigations of Macklin:

1) In aseptic brain lesions (of the nervous parenchym itself) macrophages occur, originating from the glia; they are slightly stained by vital dyes.

2) In experimental abscesses of the brain, macrophages appear, which are apparently developed from the mesenchym. They stain very brilliantly with trypan blue, much more so than the brain macrophages of the aseptic cases.

That these facts are of an eminent importance will be proved afterwards.

I may remark here however, that on account of the analogy in the distribution of vital dyes and salvarsan in the organism, it becomes more evident why in tabes and general paresis the salvarsan preparations have little or no influence, whilst they are capable of curing gummata and luetic meningitis.

The latter processes are characterized by the presence of the cells, which are described in the experimental abscesses of the brain.

In general paresis, the lues nervosa (parenchymatous syphilis of the nervous tissue), equally exsudate cells are to be seen in the neighbourhood of the intraparenchymatous spirochaetes.

In the vascular form of brain syphilis, however, more exsudate cells, originating from the mesenchym (pia), occur than in genuine lues nervosa.

Consequently we may expect that in the infiltrations consisting of mesenchymatous cells more dyes or salvarsan will be stored than in lues nervosa parenchymatosa.

In the preceding lines I have endeavoured to prove that analogies exist between salvarsan and vital dyes, especially in relation to its chemical and physical properties.

Consequently the question arises, whether something is known about the distribution of salvarsan in normal and pathological conditions, and if this distribution may be compared with that of vital dyes (e.g. trypan blue).

The indication of salvarsan in tissues, however, is very difficult, though we have two methods at our disposal viz. that of Abelin and Ehrlich-Bertheim.

The latter has been used by many student; the reagent consists of a 1 % solution of para-dimethylamino benzaldehyde in normal hydrochloric acid. This is not a very accurate method. [1]

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[1] The addition of HgCI however, increases its usefulness.

Tabel (1 drop of a solution of salvarsan + 1 drop of the reagent)

Salvarsan	without $HgCL_2$	with $HgCL_2$
0.5 %	orange	orange, precipitation
0.05 %	yellow	,, ,,
0.005 %	colourless	light orange, no precipitation
0.0005 %	,,	colourless

The method of Abelin is neither exact. The principle of this method is the diazoting of salvarsan by means of natrium nitrite and hydrochloric acid, followed by addition of resorcin. A yellow colour appears in positive cases.

Usually those methods are not used in the study of the distribution of salvarsan in the body; most investigators work with indirect methods, e.g. the demonstration of the arsenic groups.

One of these methods is that of Bohrisch and Kürschner. The liquid is distilled after addition of strong HCL, hydrazine sulphate and potassium bromide (Katalysator).

After the distillation, the arsenic is titrated with the aid of a 1/10 Normal solution of iodium.

1 c.M^3. 1/10 N. iodium = 0.004948 arsenictrioxyd = 0.003748 arsenic.

Regarding the distribution of salvarsan in the body, the following facts may be mentioned.

Ritter and Bornstein, after intravenous injections of salvarsan in rabbits, succeeded in indicating arsenic in the lungs, liver and kidneys.

In men, the latter could book analogical findings; he had the opportunity of examining the corpse of a man, who had died a fortnight after an intravenous injection of neosalvarsan (by an intercurrent disease).

He found:

liver	6,5	m.Gr. of arsenic
kidneys ..	3,1	,, ,, ,,
spleen	1,8	,, ,, ,,

As we must endeavour to explain the storage of a drug from its properties, and as we have seen (in the chapter on Goldmann's experiments) that the vital dyes are stored in definite groups of cells, and we know that salvarsan has much analogy with those dyes, its storage in the organs should not astonish us.

Salvarsan has been indicated also in the placenta.

Stümpke and Siegfried, by making use of the method of Gutzeit (formation of AsH_3), obtained curious results: Arsenic could be indicated in the urine 10—13 minutes after the injection. (This agrees with the excretion of vital dyes by the urine). As to the blood, arsenic could be indicated only the first day after the injection.

In the corpse of a child (one month of age), arsenic was found in lungs, liver and spleen; no arsenic could be indicated in brain, heart, lungs, testes, blood. (Four days after the injection of salvarsan).

They concluded from their experiments, that some organs (liver, spleen, etc.) fix salvarsan for a long time. This is in accordance with the results of Heuser and Fischer, who were able to indicate arsenic in the urine nine months after an intravenous injection of salvarsan.

Reviewing these facts, it may be said that, according to many investigators, the distribution of salvarsan in the organism is a consequence of its storage in definite organs, especially liver, spleen, etc.

In brain and cord salvarsan has never been found.

The suprarenal gland and the testes do not store it either, whilst the choroid plexus and the meninges have not been examined in this regard.

No communications are made on the cellular elements which have the property of storing salvarsan, etc.

Probably the pyrrol-cells (as described in the preceding chapters) must be considered as such, especially in connection with the analogy between salvarsan and vital dyes. Analogous cells occur abundantly in liver, spleen, etc.

The hypothesis has been stated, for instance, by the fact that fixation of salvarsan in artificial depots agrees precisely with that of vital dyes. (Downey).

Another support may be found in pathology.

We have seen that trypan blue is stored in large quantities by inflamed or dead organs. As to salvarsan analogous communications have been made.

Igersheimer, who studied the influence of salvarsan treatment on syphilis of the cornea in rabbits, found that the normal cornea did not fix salvarsan. In the syphilitic cornea arsenic could always be found after injection. He concludes.

„Es kann dieser Befund wohl gar nicht anders gedeutet werden, als dasz Arsen in der spezifisch erkrankten Hornhaut gespeichert, dasz also die wirksame Arsenverbindung von dem syphilitischen Gewebe oder den Spirochaeten selbst chemisch gebunden wurde". (p. 2676).

In the hind part of the bulbus oculi arsenic could never be indicated. (a strong proof against the so-called neurotropy of salvarsan).

Nevertheless Igersheimer believes that the influence of salvarsan is a result of its direct action on the spirochetes, in contradistinction with Lesser, who as I do, accepts the organotropy as the principal factor. He tries to prove this view:

1) by the fact that salvarsan treatment has a good influence on many non-syphilitic diseases.

2) on account of the elective action of salvarsan on pathological tissues.

The surplus of pathological tissue proves to be resorbed quickly, whereas in defects of the skin, granulations appear a short time after the injection, followed by epithelisation.

(Good results have been obtained in lichen ruber planus, psoriasis, pemphigus, verrucae, etc.)

As to syphilis, salvarsan has an excellent influence on the general state of health of the syphilitic patient, whilst the best results may be booked in ulcerative processes and gummata.

Consequently he believes that salvarsan increases the vitality of the tissue cells, thus acts especially by its organotropy.

This hypothesis may be proved by the following arguments:

1) As it is known that the the production of bactericidal substances depends on the constitution of the patient, etc. we may expect that, in marantic patients, this production will be small. Consequently, in such patients, syphilis will progress quickly and produce intense changes of skin, etc. This is a fact which has been stated by the appearance of grave ulcerative processes in the secundary period, in marantic persons (syphilis tertiaria praecox).

After the application of salvarsan, thus probably after an increased production of bactericid substances, these processes disappear quickly.

2) It is a fact that granulating wounds have a high bactericid action. As we have seen that granulating wounds especially heal quickly under the influence of salvarsan (being a consequence of the influence of salvarsan on the vitality of granulation cells), it is comprehensible that spirochaetes, living there, will be killed quickly.

3) Lutz has described cases of syphilis, which did not react to salvarsan. The patients infected by them, however, were cured totally after salvarsantreatment. An explanation of this fact may be given by accepting a disturbance in the cells of those patients, which probably are not capable of producing bactericid substances.

4) Trypan rot in vitro is not capable of killing the trypanosomes (Ehrlich).

Ehrlich has already tried to explain this by accepting that, after injection of the dye, a reaction has to occur, causing the death of the trypanosomes.

Analogous facts respecting the compounds of mercury have been mentioned by F. BLUMENTHAL.

Conclusions from this chapter.

At the end of this chapter I will shortly summarize my considerations on the inactivity of neosalvarsan in lues nervosa.

There are analogies between vital dyes and neosalvarsan, especially as regards their physical and chemical properties.

Vital dyes are fixed by definite groups of cells; probably the same may be said about neosalvarsan.

Those cells are found especially in inflammatory processes, gummata, tumours etc.

We may expect from this, that in luetic tissues (or other pathological processes), more neosalvarsan will be stored than in normal ones.

This agrees with the experimental results of Igersheimer, Lesser, etc. In general paresis, one of the forms of lues nervosa, those cells occur but seldom in the neighbourhood of the spirochetes, in contrast with gumma cerebri or luetic meningitis.

In the latter processes neosalvarsan will be stored in the neighbourhood of the spirochetes. In paralysis little or no neosalvarsan will be found there. The vitality of the phagocytic cells (in gummata, etc.) will be activated, and consequently the spirochetes will be destroyed probably by the formation of specific antitoxins.

This vital process will not occur in paralysis.

Thus the neosalvarsan treatment of lues nervosa will not be an efficacious one; although evidently the interstitial changes (likewise occuring in these diseases) will be combated, the parenchymatous alterations remain uninfluenced.

In the preceding pages I have studied especially the antiluetic drug most used, neosalvarsan. The analogy neosalvarsan — vital dye is a more striking one, than between vital dyes and the other salvarsan preparations.

All the substances of the salvarsan group are semi colloids, as are the vital (acid) dyes.

But neosalvarsan is, just as trypan blue, etc., an acid compound, having a sulpho-group in its molecule.

My considerations on cataphoresis therefore, may especially hold for neosalvarsan. I believe however, that all colloidal and semi-colloidal substances are stored in the organism in an analogous way.

And as the salvarsan substances are semi-colloids, their distribution in the body may probably be compared with that of colloidal and semi-colloidal dyes.

CHAPTER VI.

Critical remarks on the intraspinal injection as a therapeutic method, in connection with the results of my experimental investigations.

After having given in the preceding chapters a critical investigation in relation to the experiments of Goldmann, and having given (in contradistinction with his plexus hypothesis) another explanation of the fact that vital dyes do not penetrate into the nervous system, I shall endeavour in this chapter to give my views on intraspinal treatment, especially in tabes and general paresis.

This chapter will be divided after the following scheme:

1) On the quantity of salvarsan applied endolumbally, and on its spread over the surface of the central nervous system.

2) On the influences acting upon injected salvarsan, which, by changing its properties, diminish the action on the syphilitic processes.

3) On the diffusion of salvarsan, in connection with its penetration into the nervous tissue.

4) On the dangers of intraspinal treatment.

1) Quantity and spread of the injected salvarsan:

In chapter IV I have given a description of the spread of dyes after intraspinal injection. Trypan blue proved to be fixed by pia cells of the spinal cord and the base of the brain.

The convexity of the brain was not reached by the dyes.

In general paresis, a disease where the pathological process is localized in the cortex cerebri and especially in the frontal part of it, (the spirochetes being accumulated in this part of the nervous system) it is intended to apply salvarsan to that place.

Apart from the question whether the presence of salvarsan in the neighbourhood of the spirochetes is sufficient to kill them, the following question presents itself:

If injected intraspinally does salvarsan reach the convexity of the brain?

As I have demonstrated in the preceding chapter that an analogy exists between salvarsan and vital dyes, I believe we may compare the spread of vital dyes over the surface of the nervous system with that of salvarsan.

From this I may conclude that, after intraspinal injection of salvarsan, no drug reaches the frontal lobes.

If we accept, with Lewandowsky, that the liquor cerebrospinalis is a stagnant fluid, we may expect the greater part of the injected salvarsan to remain in the immediate neighbourhood of the place of injection.

The injected salvarsan will spread by diffusion, until a further spread is made imposible by resorption (vital storage or adsorption). If large doses of the dissolved material are injected, it will probably penetrate into the ventricles, as results from my experiments.

But, even though these experiments have been made with large doses of vital dyes, no dye could be indicated on the convexity of the brain.

Consequently we may expect that the intraspinal treatment of general paralysis will be followed by negative results.

Lafora affirms that after intraspinal application of dyes in animals, the convexity of the brain is not reached.

He believes, however, that in men other relations exist:

„car les injections intrarachidiennes de préparations mercurielles et salvarsaniques produisent bientôt des phénomènes accusateurs de l'action irritante des préparations sur les centres corticaux de la convexité (troubles de la parole, nausées, cephalalgie, etc." (pag. 627)

Those facts may be interpreted in another way, since it is known that the headache appearing after the injection of large quantities of liquid, is not always the result of a direct toxic action on the convexity of the brain, but may depend on an increase of the endocranial pressure.

The nausea and the troubles of speech may result from a direct action on the medulla oblongata (cranial nerves: vagus, etc.)

Thus the arguments of Lafora may not be considered as very conclusive. As to the doses of salvarsan applied, I communicated in chapter III that generally I or 2 m.Gr. are injected intraspinally. If we may accept that this quantity is sufficient to yield therapeutic results, the question arises as to how much of it attains the diseased region.

2) Influences acting on injected salvarsan.

As having an influence on the result of injected salvarsan, the following factors may be considered:

a) Fixation of salvarsan by definite groups of cells.

b) Fixation of salvarsan by dead cells.

c) Adsorption of salvarsan to substances dissolved in the cerebrospinal fluid.

d) Changes in the physico-chemical properties of salvarsan by the action of H-ion concentration, etc.

Ad a) In the preceding chapters (IV, V.) I have mentioned the fact that trypan blue (either after intravenous or intraspinal injection) is stored by definite groups of cells, e.g. the pyrrol cells, interstitial cells of the connective tissue, etc.

And as I have demonstrated the analogy between salvarsan and vital dyes, it is probable that a part of the injected salvarsan will be stored as „salvarsan granules" in the protoplasm of the cells of the connective tissue of the meninges.

In neurosyphilis the number of those cells has greatly increased, (by inflammation) from which it follows that the quantity of stored salvarsan will be higher than under normal conditions.

Also the cells of the liquor cerebrospinalis have increased in neurosyphilis, and as Downey has demonstrated that these cells, in definite conditions, may store vital dyes, we may assume that they also store a part of the applied salvarsan.

Probably this fixation is a temporary one: the stained cells will (under the influence of the stored salvarsan) be induced to a higher activity, and consequently, the spirochetes which are accumulated in liquor or meninges, will be killed.

As we may, basing on the analogy between salvarsan and vital dyes, assume that salvarsan will be stored in the connective tissue cells of the meninges, the intraspinal treatment is not absolutely necessary.

Ad b) Dead cells are capable of fixing vital dyes to a high degree (see chapter V).

My own experiments could prove this fact, whilst Stümpke and Siegfried, in an abscess, caused by the injection of salvarsan, succeeded in indicating more salvarsan in the necrotic tissue than in the abscess cavity itself.

In lues nervosa many dead cells (ganglion and glia cells) may be demonstrated in the central nervous system. Evidently they will fix a great deal of the injected salvarsan.

It results from Jahnel's microscopical studies that, in general paresis the spirochetes occur especially in the neighbourhood of the living cells.

If we accept the theory that a spirocheticidal action of salvarsan is only possible by its presence in the neighbourhood of the spirochetes, the salvarsan stored in the necrotic tissue will have little or no influence on the syphilitic process, firstly owing to its fixation in this necrotic tissue, and secondly to the absence of spirochetes in the neighbourhood of the dead cells.

Ad c) Of the adsorption of salvarsan to substances dissolved in the cerebrospinal fluid no facts are known.

That this process is of great importance in intraspinal treatment and may be neglected in intravenous application of salvarsan, results from the following considerations:

If we apply a drug intraspinally, it is introduced into a space where few or no currents occur. Thus, practically, salvarsan is introduced into a stagnant fluid. (according to Lewandowsky).

This stagnant solution of salvarsan will be influenced by its solvent. And since the spread of salvarsan over the nervous system is probably the result of mere diffusion in a column of fluid, it will be possible that, if this fluid is a solution of colloids (albumen, etc.), adsorption of salvarsan may occur to these colloids.

Consequently less salvarsan than is introduced into this fluid can act upon the nervous system.

Gradually, however, part of the adsorbed salvarsan will be released, but a part of it remains in state of adsorption.

Especially Bechhold considers the adsorption of drugs to dissolved proteins, as an important fact.

He believes that it will be possible (by making use of his method of ultrafiltration) to study the laws of distribution of dissolved colloids and crystalloids.

Since the investigations on adsorption are made especially with powders (charcoal, etc.) and hydrophobe colloids as adsorbentia, and we know that in the organism especially hydrophil colloids occur, it would be of eminent importance if analogous investigations were instituted with the aid of hydrophil sols. (albumen, protein, globulin, etc)

Few communications have been made on this object: Bechhold himself studied the distribution of methylen blue between water and serum albumin.

It appeared that highly diluted solutions of methylen blue are fixed for the greater part by albumen, whilst with increasing concentrations the distribution is altered in such a sense that the water contains more of it.

Probably the curve may be considered as a common adsorption curve.

Since in intralumbal treatment small quantities of salvarsan are introduced into the cerebrospinal fluid, and it is known that, as caused by he syphilitic processes, the amount of globulin in the liquid has highly increased, it may be assumed that part of the injected salvarsan will be fixed to the dissolved globulin.

Probably this will be one of the reasons why the therapeutic action of salvarsan on those processes will be less than has been expected. Investigations on the adsorption of drugs to normal hydrophil colloids of blood and other fluids of the body will probably be able to give us an explanation of many facts which have not hitherto been understood.

Ad d) The same facts as mentioned sub c, must be taken into consideration before we give a description of point d.

We inject salvarsan into a column of fluid and make a highly diluted solution of salvarsan in the liquor cerebrospinalis.

The question arises whether there may be factors which change the properties of dissolved salvarsan.

From the experiments made with the substances of the salvarsan group, we may conclude that the dispersity of salvarsan decreases by addition of small quantities of acids.

We may ask in this connection, if slight changes in the H-ion concentration have an analogous influence on the dispersity of semi colloids, of which salvarsan is an example.

Höber mentions facts which plead for this hypothesis:

„namentlich wird mehr als bisher zu berücksichtigen sein. dasz oft mit geringfügiger Abweichung der Reaktion vom Neutralpunkt nach der sauren oder nach der alkalischen Seite eine Dispersionsverringerung Hand in Hand geht". (p. 427).

The investigations of Wolfgang Ostwald entirely agree with it. The H-ion concentration of blood is about the same as that of cerebrospinal fluid. (Höber).

According to Bornstein, the alcalescency of the blood of general paretics has decreased; probably the same may be expected of the cerebrospinal fluid.

In the fluid of patients with lues nervosa the H-ion concentration, thus, may show an increase which probably will reduce the dispersity of introduced salvarsan.

Few facts are known about those problems, and generally little attention has been paid to the influence of H-ion concentration on applied drugs, an influence which may not be overlooked, especially since there is a tendency to prepare chemotherapeutical preparations of a high molecular weight.

The same considerations may be studied for the organotherapeutic substances. They equally will be influenced by „geringfügiger Abweichung der Reaktion vom Neutralpunkt nach der sauren oder nach der alkalischen Seite".

An increase of the degree of acidity will, a priori, have a greater influence on the dispersity of colloid and semi-colloid drugs than an increase of the OH-ion concentration.

In this regard some communications may be made on silversalvarsannatrium.

Ultramicroscopically in the solutions of this material no particles are to be seen.

The solution does not show the Tyndall phenomenon.

If, however, a small quantity of acid is added, red particles immediately become visible, and the solution changes into a cloudy one.

Consequently we may conclude that the H-ion concentration has a great influence on the dispersity of semi-colloids.

„Man wird an die koagulierende Wirkung erinnert, die Säuren auf kolloide Substanzen ausüben". (Bauer).

Since the diffusion of drugs and dyes depends on the size of their particles (molecular aggregations), and it is necessary to make the rate of diffusion of intraspinally applied drugs as high as possible, it is comprehensible that the decrease of the dispersity (as a result of the increased degree of acidity) does not favour our therapeutic results.

In the following lines I will endeavour to elucidate the importance of diffusion, as a condition of the action of intraspinally applied drugs on the central nervous system. (in lues nervosa).

III. On the diffusion of salvarsan into the nervous tissue.

We may conclude from the facts mentioned above (sub a, b, c, d), that only a small part of the injected salvarsan (intraspinally), can have a therapeutic action on the nervous system.

The question then arises whether the unchanged residu of the salvarsan (supposing its distribution through the liquor cerebrospinalis), may penetrate into the nervous tissue itself. This question is of great importance in connection with my opinion on the lymph-circulation in the central nervous system itself. (Chapter I)

I assumed a lymph space surrounding the vessels and capillaries of the nervous tissue, Virchow-Robin's sheath. Dissolved drugs, introduced into the arachnoid cavity, will not be in direct contact with the ganglion cells themselves.

If, however, the dissolved materials have a high rate of diffusion, they will be capable of passing the membrana limitans gliae and reaching the ganglion cells. This may be said about crystalloids, such as sugar, salts, etc.

Substances, with a high molecular weight, such as salvarsan, and having a low rate of diffusion, will not be capable of passing the membrana limitans and reaching the ganglion cells, unless they destroy the tissue and consequently change its properties.

The diffusion of drugs and dyes into gelatine may be considered as being in proportion to their diffusibility.

Drugs, with a high rate of diffusion, will be capable of penetrating into the nervous parenchym itself (the ectodermal part of the nervous system), drugs with a low rate of diffusion will not be able to do so.

Diffusion experiments with salvarsan, etc. have been described, whilst I myself have made preliminary investigations on this subject. (not neglecting to take into consideration the influence of H-ion concentration, etc. on the rate of the diffusion).

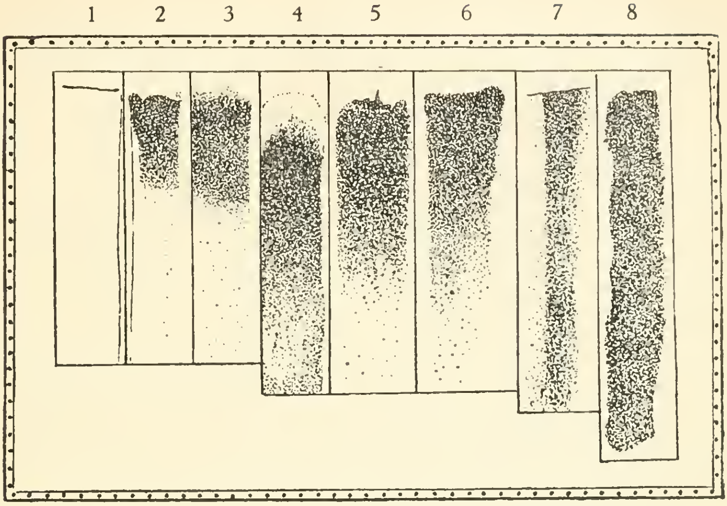
Bauer (see chapter V) studied the diffusion of the salvarsan into gelatine. (fig. 10).

According to their rate of diffusion, the drugs studied may be arranged in the following list, in which the slowest diffusion is found in collargol.

1. Collargal.
2. Sulfoxylat.
3. Silversalvarsannatrium.
4. Neosalvarsan.

5. Salvarsan-dichlorhydrate.
6. Salvarsan (alcaline).
7. Salvarsannatrium.
8. Atoxyl.
9. Silvernitrate.

Fig. 10.



Diffusion of salvarsan, etc. into gelatine (BAUER).

1 = Collargol
2 = Sulfoxylat
3 = Silversalvarsannatrium
4 = Neosalvarsan (the under part of the reproduction is too dark)
5 = Salvarsan (in alcaline solution)
6 = Salvarsannatrium
7 = Atoxyl
8 = Nitras argenti

Consequently we may conclude that, if salvarsan is introduced into the liquid, its penetration into the nervous parenchym will be a slow one.

The question now arises whether the rate of diffusion of salvarsan, etc. is influenced by the solvent used.

Preliminary investigations on this subject have been made; they have not succeeded, however, on account of the precipitation of the salvarsan solutions employed.

A description of my scheme may, however, be given.

I made solutions of:

Neosalvarsan in normal saline solution.
,, ,, aq. dest.
,, ,, normal serum.
,, ,, ,, ,, + acid.
,, ,, ,, ,, + alcali.

The diffusion of those solutions into gelatine was studied, by following a way similar to Bauer's.

It would be of eminent importance to repeat those experiments with the utmost precaution: the action of the oxygen of the air must be excluded. Then it will be possible to study the diffusibility of salvarsan under the influence of different solvents.

It will be necessary to use as solvents normal liquid, liquid and serum from general paretics, etc.

In intraspinal treatment analogous factors influence the dispersity of salvarsan, and thus its degree of penetration into the nervous parenchym.

Some neurologists for instance, inject solutions of salvarsan in serum, (salvarsanized serum), which probably may not be considered as a common solution, but as a solution on which adsorption has great influence.

Conclusions: I am inclined to believe that the intraspinal application of drugs is not more preferable than their intravenous application. For, if the injected materials have a high rate of diffusion, intravenous as well as endolumbal application may cause their penetration into the nervous tissue. (If their presence in the liquid is a condition of the penetration into the nervous tissue, has been discussed in chapter I).

If the injected drug has a low rate of diffusion, the intravenous application will be just as efficacious as the endolumbal one.

In the latter case the specific elements, which are capable of storing the drug, will be attained as well as by following the former way.

Dercum equally considers the intraspinal method as an unefficacious one .He assumes that substances introduced into the cerebrospinal fluid rapidly disappear by passing out through the arachnoidal villi (Chapter I) without in the slightest degree penetrating the nervous parenchyma.

That the direct injection of semi-colloids (salvarsan, etc.) into the nervous tissue in small doses, does not offer hopeful prospects for the therapy of lues nervosa, may be concluded from chapter IV. After intracerebral application of trypan blue the spread of this dye in the nervous system proved to be insignificant.

IV. After this review of the theoretical objections to intraspinal treatment, I will describe in the following lines some more practical difficulties in this respect.

A. Dangers of intraspinal treatment (in animals.)

As far as concerns the influence of salvarsan on the nervous elements (after direct application in animals), many experiments have been made.

No final conclusions may yet be drawn, though it seems that salvarsan (even in therapeutic doses) has not a good influence on the nervous elements.

Camus was the first to inject solutions of salvarsan into the arachnoid cavity of dogs.

After intraspinal injection of $^1/_4$ c.c. of 1 % solution of salvarsan in a dog, the gait was altered. The animal died the third day after the injection (showing convulsions, etc.)

He concludes from his experiments:

„Il me paraitrait hasardeux d'introduire des doses même faibles de ces substances dans le canal rachidien de l'homme.

Sans doute une injection faite par voie lombaire peut rester dans la partie inférieure des méninges et ne pas diffuser jusqu'aux centres supérieurs.

Mais on risquerait que cette diffusion se fasse, et alors un tableau symptomatique analogue à celui que j'ai observé chez le chien pourrait se produire."

Swift and Ellis made use of monkeys for those experiments; intraspinal injection of 3 m.Gr. of salvarsan caused paralysis of the hind legs, and pleocytosis of the liquid.

Castelli never observed any bad results. Even if salvarsan was applied directly on the conjunctiva of rabbits, no symptoms of irritation arose.

Since those authors did not agree on this subject, Berger repeated these experiments by following another method of investigation.

He made use of the method of Hill: irrigation of the arachnoid cavity. (See Chapter III).

The neosalvarsan was dissolved in a 0.4 % solution of NaCL of 22° C., and the injection was made under low pressure.

Doses of 3 m.Gr. caused the death of the animal after a period of strong convulsions.

Infiltrates and hemorrhages were invariably observed in the nervous tissue and meninges.

Berger ascribes these changes to the direct action of salvarsan upon the vessels of the nervous system and meninges. Since he saw that the followed method, as such, is capable of causing changes in the nervous tissue, he also made use of the direct injection into the arachnoid cavity.

The results were the same (convulsions, death), whilst the autopsy again showed infiltrates and hemorrhages.

By injecting only 0,1 m.Gr. of neosalvarsan, the symptoms, etc. could be avoided.

From his experiments, Berger concluded that the concentration of the solutions used is an important factor. „Die Konzentration der Neosalvarsanlösung darf nicht über 1 : 1000 hinausgehen, da sonst lokale Veränderungen an der Injektionsstelle in der Form von Infiltraten der Meningen und Veränderungen der Pial-und Rindengefäsze sich einstellen." (p. 353).

With monkeys analogous experiments were instituted by Weygandt, Jacob and Kafka.

Intraspinal injection of salvarsan caused alteration of the dura near the place of injection, degeneration of the spinal nerves, ganglion cells, etc.

Subdural injection (into the cranial cavity) likewise caused degeneration of the endothelium of the vessels.

Myslivecek and Willige obtained analogous results; the latter especially drew attention to meningeal reactions (pleocytosis, etc.).

From the facts mentioned above, we may conclude that the application of salvarsan on the nervous system, via the liquor cerebrospinalis, has a bad influence especially on its vessels.

Also he parenchym itself is altered.

A knowledge of those facts must urge us to consider the intralumbal injection of salvarsan as a dangerous method, though the alterations in the nervous tissue do not always cause clinical symptoms.

Another question, which may be of importance in connection with the antiluetic therapy of neurosyphilis, is he fact, that the action of salvarsan on injured nervous tissue is still more dangerous than on normal tissues.

Especially the researches of Luithlen, who saw that after X-ray treatment of the brain, intravenous injections of salvarsan often caused an encephalitis haemorrhagica, have made us prudent in this regard.

Especially the intracranial injection should be avoided, since by making use of this method lesions of the brain tissue often occur.

In the following pages I will endeavour to prove that the direct application of salvarsan on the nervous tissue in men, may also be followed by serious symptoms.

That the injection of mercury, etc. into the arachnoid cavity must likewise the considered as a dangerous method, may be concluded from the experiments of Camus, Myslivecek, and others. (anatomical lesions of the nervous tissue after the intraspinal application).

B. Dangers of the intraspinal treatment in men.

We have seen in chapter III, that the therapeutic results obtained by the intraspinal method must be considered as being inefficacious.

If a method is a dangerous one, we may only recommend it when its therapeutic results outweigh the more nocuous effects.

This cannot be said of the intraspinal method, for its therapeutic results do not outweigh the dangers that menace the patient. Many communications have been published on the dangerous results of this method.

Gordon for example mentions the following case. A tabetic had been treated by means of intraspinal injections of salvarsanized serum.

After the second injection intense pains in the lower extremities occurred, followed by vomiting, retentio urinae, etc.

An exantheme could be observed, which resulted into gangrene, death following quickly.

Zadek describes an analogous case:

A tabetic was treated with intraspinal injections of neos l-varsan (0.0036 Gr.)

Two days after the injection the patient died in deep coma, showing Cheyne-Stoke's respiratory phenomenon.

He believes that this case must be considered as an encephalitis haemorrhagica, caused by the direct action of salvarsan on the nervous tissue.

The results of Read were equally discouraging. In one case he observed an increase in the spastical symptoms, three patients showed incontinentia urinae, whilst some complained of pains and paraplegia.

Marinesco, who applied salvarsan endocranially, mentions bad results: three out of 17 patients died, as a direct result of this treatment. [1]

Finally I will describe some results obtained in the Psychiatric Neurological Clinic of the University of Amsterdam.

Though the therapeutic method followed here, was the same as in other clinics, the results were rather unsatisfactory.

Nevertheless it will be useful for other investigators, who believe in this method, to read the descriptions of the cases treated.

C. Clinical cases, observed in the Wilhelmina Hospital.

1. A. van V., born 11 May 1875. Taboparesis.

3-2-'17 Previous history.

Patient suffers from increasing dullness. His memory is bad. For a year his gait has been altered and speech hesitating and clipped. Patient has lost control of the bladder. A cutaneous hyperaesthesia of hands and feet exists. Girdle pains. In his youth patient has contracted syphilis and gonorrhoea and has taken much strong drink .

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[1] In a critical review on the intraspinal treatment of lues nervosa, Marcorelles describes the dangers of this therapeutic method. He observed after the administration of salvarsan lancinating pains, retentia urinae, paraplegia, etc. He ascribes these bad results to the action of salvarsan on the nervous tissue, which has lost much of its natural resistence.

Neurological Examination: patient is quiet and is not disorientated.

Patient has lost the use of his finer faculties. His memory is failing.

The pupils are unequal, the left being larger than the right one.

The left pupil reacts on light and accommodation, the reaction of the right is a sluggish one.

The knee-jerks are exaggerated.

Speech: hesitating.

Spastic-paretic Gait: unsteadiness in walking. He plants his feet widely apart.

Romberg's sign is positive.

Tremors of tongue, facialis, hands and legs.

Cerebrospinal fluid: W.R. = 10/10
Nonne = +
Pleocytosis = 41

Blood: W.R. = 6/10.

THERAPY: Pat. is treated with intravenous injections of neosolvarsan.

Within 8 weeks 4500 m.Gr. are injected intravenously.

18 June 1917: Intraspinal injection of 3 m.Gr. of neosalvarsan. After the injection: progressive increase of ataxia.

The urine escapes involuntarily.

27 Oct. 1917: The pupils are unequal, but react to light and accommodation. The knee-jerks are exaggerated, ankle-clonus.

8 Nov. '17: Intraspinal injection of $1^{1}/_{2}$ m.Gr. of neosalvarsan.

25 Nov. '17: Intraspinal injection of 2 m.Gr. of neosalvarsan.

The incontinence of urine is increasing quickly.

The gait gets quickly worse.

18 Dec. '17: Paralysis of both legs: it is possible to elicit involuntary movements by pricking the legs.

Contracture of both peroneal muscles.

The incontinence has increased to a high degree.

Anaesthesia of the under part of the body: the upper limit of the hypaesthesia passes through the umbilicus.

In this region neither has he tactile sensation, nor feels pain, etc.

Total loss of the sense of position of the limbs.

The plantar reflex shows at both sides Babinski's typus.

Knee-clonus.

21-2-'18: The psychical state has not changed.

In the lower extremities there is a spastic paraplegia.

Anaesthesia up to a line passing through the umbilicus. Bed-sores of the nates.

28-2-'18: Patient vomits and complains of pain in the abdomen. Temperature: 38.4° C.

1-3-'18: Cyanosis — The abdominal walls are tender to pressure and retracted.

Death of the patient.

Post mortem diagnosis: Atrophia cerebri, transverse myelitis, ulcerative endocarditis.

Anatomical description:

The cord, after embedding in celloidin, is cut into sections, which are stained with Weigert-Pal and von Gieson methods.

In the sacral region a typical degeneration of the posterior columns is noticeable.

The atrophy is not universal, the cornu-commissural zones being intact.

The area Lissauer is degenerated, as well as the posterior roots. No degeneration of the cortico-spinal tracts. The anterior roots, on the contrary, show degenerative changes.

There is an intense thickening of the pia (fig. 11). The vessels are affected.

In the pia many lymphocyte infiltrates are visible; in some parts of it the infiltrates are so great, as to be described as purulent processes.

There is also lymphocyte infiltration in the posterior and lateral columns and in the grey matter. The anterior roots are totally embedded in the inflammatory tissue of the meninges. The motor cells of the anterior horns are intact.

In the lumbar region the degenerative and inflammatory changes also occur, even to a higher degree. Many infiltrates are visible in the anterior columns.

In the dorsal part of the cord, also the cornu-commissural zones are intact. The pyramidal tracts show infiltrates, but no degenerative changes.

In the cervical region the inflammatory changes in the cord itself have diminished. The degeneration, etc. is as in the sacral region.

2. Jan. D, aged 43. (Taboparesis)

17-7-'17. Patient is admitted to the hospital, since he has showed troubles of the speech, altered gait, lancinaing pains and defects of the memory.

Gonorrhoea, no syphilis.

Neurological Examination:

Tremors in hands and legs, especially on the right side.

Hypalgesia of the whole body; the upper part of the back proves to be analgetic.

Disturbance in the deep sensibility of the legs. Ataxia.

Gait: atactic. Romberg's sign is positive.

The pupils are equal, do not react to light. Good reaction to accommodation.

Absence of knee and ankle-jerks.

Psychical state: Retention and memory show defects.

Cerebrospinal fluid: Wassermann R. 9/10
Nonne +
Pleocytosis 42
Blood: Wassermann test 8/10

23-7-'17: Patient is treated with intravenous injections of neosalvarsan. Within 7 weeks 4500 m.Gr. are injected intravenously.

26-9-'17: The incontinence of urine is increasing quickly; the ataxia of the gait also increases.

31-10-'17: The psychical state inclines to get worse: patient loses his sense of judgment and shows typical megalomania.

7-11-'17: Intraspinal injection of $1^{1}/_{2}$ m.Gr. of neosalvarsan.
8-11-'17: Temperature is 38.2 C.
13-11-'17 Intraspinal injection of 2 m.Gr. of neosalvarsan.
20-11-'17: „ „ „ 3 „ „ „
The incontinence has increased to a high degree.
28-11-'17: Intraspinal injection of 1 m.Gr. of neosalvarsan.
6-12-'17: „ „ „ 2 „ „ „
13-12-'17: „ „ „ 2 „ „ „
20-12 '17: „ „ „ 3 „ „ „
26-12-'17: „ „ „ 3 „ „ „
9- 1-'18: Patient gets worse and worse. Hallucinations. Paraplegia of the lower extremities. Bed-sores.
28- 1-'18: Death.

Clinical Diagnosis: Taboparesis.

Anatomical Diagnosis: Pyelitis and cystitis purulenta. Decubitis sacri and myelitis purulenta. (a direct communication was to be seen between the bed-sores and the arachnoid cavity).

Microscopical examination of the cord: Sections through the cord on different levels are stained by the Weigert-Pal, von Gieson methods, etc.

The most intense changes occur in the cauda equina. In the Weigert preparations the roots are stained very pale. They are surrounded by many infiltrates. (fig. 12).

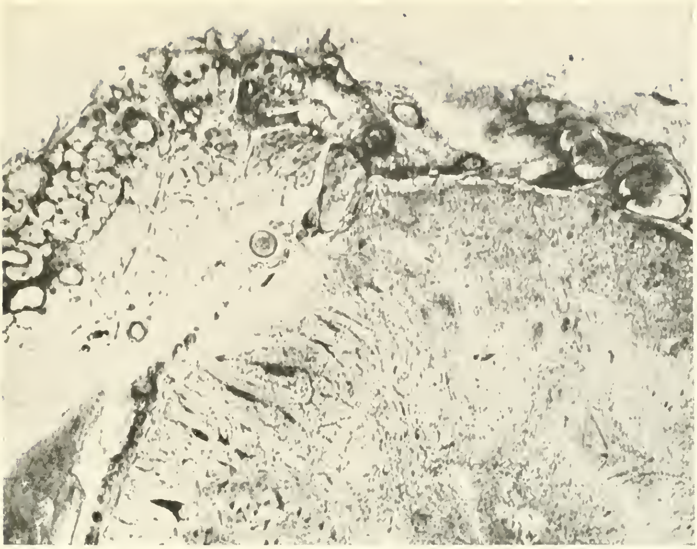
In the sacral region the posterior columns are degenerated, except the ventral part of the cornu-commissural zone. The posterior roots likewise show degenerative changes, the anterior and lateral columns are intact. The anterior roots are normal. There is a typical meningitis, with many infiltrates (here and there pus is visible).

In the cord also infiltrates are to be seen, but fewer than in case No. 1.

Lumbar region: slight atrophy of the right pyramidal tract. No inflammatory changes may be observed in the cord itself.

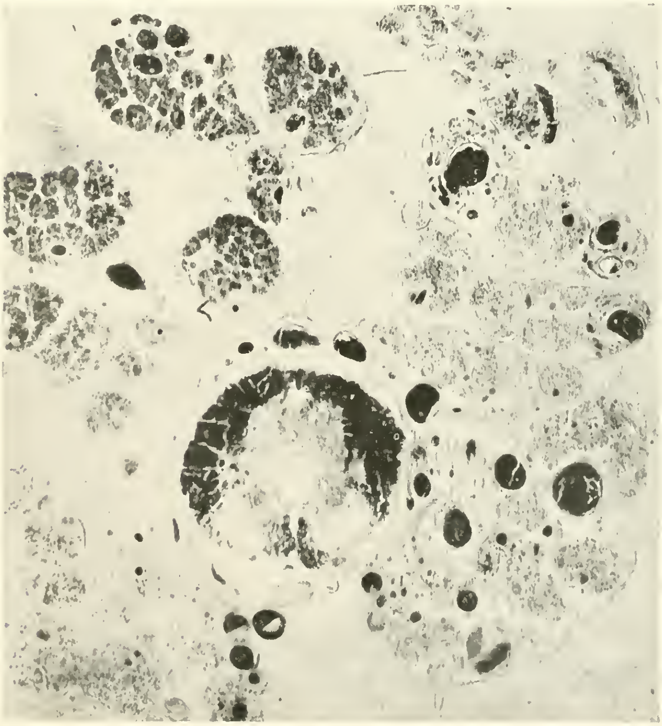
Thoracic region: atrophy of both pyramidal tracts. Degeneration of the posterior columns. The posterior roots are slightly affected. The anterior roots are intact. The meningitic process is, at this level, clearly visible. Little inflammatory changes of the cord itself.

Fig. 11.



CLINICAL CASE N⁰. 1.
Taboparesis with meningo-myelitis, caused by intraspinal injection of Neosalvarsan.

Fig. 12.



CLINICAL CASE N⁰. 2.
Taboparesis with meningitis of the cauda equina, caused by intraspinal injection of Neosalvarsan.

Cervical region: the degeneration of the posterior columns is less intense.

The cornu-commisural zone is quite intact. The posterior roots do not show any alterations and do not vary in depth of staining.

Slight degeneration of the pyramidal tracts.

The meningitic changes are less pronounced than in the caudal parts of the cord. No inflammatory changes of the cord itself.

3. Angenita H., aged 58.

6 Oct. '17: Previous history of the patiënt:

Patient has become dangerous for her family, and is not capable of doing her work. She has lost control of the bladder, has girdle sensation and lancinating pains.

No miscarriages. Has been married twice. Her first husband died of phtisis pulmonum.

Present state: Patient is dull and apathetic. The memory is mediocre, retention fairly good. The right nasolobial fold is obliterated, the right angle of the mouth droops. Ataxia of the arms and legs (inger-nose test, etc.).

The gait is atactic, Romberg's sign is positive.

Ataxia of speech.

Pupils: the right is larger than the left one.

No reaction to light. The reflex of convergence is intact.

Tendon reflexes: Loss of the Achilles reflexes and knee-jerks. Babinski reflex at the left.

Cerebrospinal fluid: W.R. 10/10

Blood: W.R. +

Intraspinal treatment:

12-11-'17: Intraspinal injection of $1^{1}/_{4}$ m.Gr. of salvarsan.
26-11-'17: ,, ,, ,, $1^{1}/_{2}$,, ,, ,,
3-12-'17: ,, ,, ,, 2 ,, ,, ,,
10-12-'17: ,, ,, ,, 2 ,, ,, ,,
Patient complains of tingling and numbness in the legs. Paresis of both legs.

13-12-'17: Patient complains of lancinating pains in the legs. There is a muscular paralysis of the legs. The gait is worse.

17-12-'17: Intraspinal injection of 3 m.Gr. of neosalvarsan.
Pain in the legs.

21-12-'17: Incontinence of urine.

7- 1-'18: Bed-sores.
Tremors of the tongue, paraplegia of the legs, incontinence, pains in legs and abdomen, anaesthesia and analgesia of the legs.

1- 3-'18: Septichaemia. Death.

Clinical Diagnosis: Tabes dorsalis.
Myelitis transversa.

Anatomical Diagnosis: Atrophia cerebri.
meningitis chronica cerebri.
leptomeningitis myeli et decubitus.
myelitis transversa.
aortitis luetica.

Microscopical Examination of the Cord: Staining by the methods of Weigert-Pal, von Gieson, etc.

Sacral region: degeneration of the posterior columns and of the pyramidal tracts.

The depth of staining of the posterior roots is normal.

Slight meningitic changes occur. No inflammatory alterations of the cord itself.

Lumbar region: degeneration of the posterior and lateral columns. Meningitis.

Thoracic region: the typical image of a combined degeneration. In the pia perivascular infiltrates are visible.

Cervical region: degeneration of all tracts, diffuse marginal sclerosis.

In the cord itself here and there inflammatory changes are visible.

4. L., woman, aged 39. Taboparalysis.

Neurological Examination: (21 Oct. '17).

The pupils show a slow reaction to light, they are unequal and angular.

Absence of knee- and ankle-jerks. Lancinating pains. Girdle-sensation. Ataxia of speech, troubles of writing.

Reactions: Blood: W.R. 4/10
Cerebrospinal fluid: W.R. 8/10
Nonne: +
Pleocytosis: 15.

Psychical state: Has made a tentamen suicidii, has hallucinations, is aggressive and ecstatic.

Intellect: memory is bad, retention shows defects, is disorientated, shows anxious depression. (nihilistic delusions).

Treatment:

In November, patient has bed sores, followed by an abscess on the sacrum. An incision is made.

Intraspinal injection of (totally) 6 m.Gr. of neosalvarsan.

In January 1918 patient showed incontinentia urinae. Now and then incontinentia alvi.

February: lancinating pains in the legs.

The psychical state changes completely: the confusion disappears. She begins to work a little. Is very picus. The gait improves gradually. When she leaves the hospital (24-10-'18) patient walks much better than half a year ago. Psychically her state has improved, so that she is capable of doing her work again.

5. K., woman, aged 53.

Taboparesis.

Is admitted to the hospital at the 28th of August 1917:

Present state:

Ptosis, analgesia of the whole body. Romberg's sign is positive. Ataxia of speech.

Argyll-Robertson's phenomenon.

Reactions: cerebrospinal fluid: W.R. 10/10
Nonne: +
Pleocyt. 26.
Blood: W.R. 8/10.

Psychical state:

Megalomania, elaborating on sexual themes. Disturbance of retention and memory. Disorientation in time. Troubles of judgment. Illusions and hallucinations.

Treatment:

Intramuscular injections of Enesol (each day).

Intraspinal injection of neosalvarsan (total 31.5 m.Gr. are injected, with intervals of one or two weeks).

In September 1917 patient complained of incontinentia urinae.

The treatment proved to have good influence on the paretic process:
W.R. blood 7/10
W.R. fluid 8/10.

The maniacal exaltation disappeared. Finally patient was quiet and could form a good judgment of her disease.

No megalomanic ideas.

Memory and retention improved.

In 1919 patient was psychically intact.

In 1920 she had irregularities in the gait.

6. S., woman, aged 42. (Taboparesis).

8-11-'17. Is admitted to the hospital.

Previous history: For twelve years she has complained of lancinating pains in the legs. Has had an epileptiform attack. Incontinence of urine. One miscarriage. No syphilis, etc.

Present state (neurological examination):

Incontinence of urine. Hyperaesthesia of the lower part of the body. Analgesia of the whole body, paraesthesia of the feet.

Argyll-Robertson's phenomenon. Ataxia (finger — nose test). Ataxia of speech. Absence of Achilles-reflexes and knee-jerks.

Psychical state: Speech is hesitating. She is orientated. Attention is good, memory shows no defects. Retention is bad. Reading and writing are bad. Judgment shows defects.

Reactions: Blood: W.R. 0.7
Cerebrospinal fluid: W.R. 0.9
Nonne: +
pleocytosis: 51.

Treatment: Intraspinal injections of neosalvarsan, combined with intramuscular injections of sublimate.

19-11-'17 1 m.Gr. of neosalvarsan.
26-11-'17 2 „ „ „
3-12-'17 2 „ „ „
10-12-'17 2 „ „ „
17-12-'17 3 „ „ „
24-12-'17 3 „ „ „
31-12-'17 $2^{1}/_{2}$ „ „ „
31 January '18: Leaves the hospital.
The state of health is unchanged.

February '18: paraparesis. Incontinence of urine. Babinski's sign. Atactic gait.

These symptoms disappear completely. (probably myelitis transversa).

October 1920: Is admitted again into the hospital. Paralytic attacks.

From the observations mentioned I believe to may conclude that the intraspinal treatment of Lues nervosa as a therapeutic method, is an unefficacious one.

In two of the six cases we have seen some improvement (as to the psychical state). The other cases, however, prove that the intraspinal method is a dangerous one.

And since a therapeutic method may not be a nocuous one, and its results are not in relation to the difficulties, etc. of the endolumbal application, I believe to be compelled to consider the intraspinal treatment of lues nervosa as being of little therapeutic value.

Summary and Considerations on the future of antiluetic therapy in Lues nervosa. [1]

Many investigators, accepting the hypothesis of Goldmann, who considered the choroid plexus as the protecting membrane of the nervous system, ascribed to this membrane the negative results of antiluetic treatment in lues nervosa.

On account of this writer's experiments, they believed that intravenously injected salvarsan could not penetrate into the nervous tissue, a consideration supported by the impossibility of indicating salvarsan in the cerebrospinal fluid.

As a consequence of those facts, intraspinal injections of salvarsan were made.

In this relation two questions arose:

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[1] Lues Nervosa, the general name for those syphilitic diseases of the nervous system, which are characterized by a parenchymatous degeneration of the nervous tissue. (Sormani, Head).

1) Does a communication exist between the arachnoid cavity and the periganglionic spaces?

2) If salvarsan penetrates into the nervous tissue, may we then expect a direct spirocheticidal action?

The first question has been answered in the negative. (see chapter I and IV).

The second question has been studied in chapter V, where a negative answer had also to be given.

An organotropic action (demonstrated among others by Lesser, Uhlmann, etc.) had to be assumed, viz. that salvarsan was stored by definite organs and that consequently the spirocheticidal activity of their cells increased.

The negative answers to the above questions, led me to consider the intraspinal treatment as a doubtful method.

Still, many authors were inclined to inject salvarsan intraspinally, in order to avoid the protecting membranes of the nervous system.

Thus the experiments of Goldmann have greatly influenced the development of intraspinal treatment.

Every communication on this method (as a therapeutic one), was preceded by an argumentation, based on his publications.

Gradually the intraspinal treatment came to be used as a common routine method. Critical remarks, however, were not lacking (see chapter II), and as the importance of a repetition of Goldmann's experiments increased, I repeated them.

The following results were obtained:

1) If acid dyes are injected intravenously, vital staining may be observed in definite groups of cells (reticulo-adventital cells) (see chapters II, IV).

Also dead cells fix the dyes to a high degree. Their staining is diffuse. These results agree with Goldmann's.

2) The results obtained after intraspinal injection of those dyes, however, differ widely from those of Goldmann. He had described symptoms of nervous irritation, which appeared immediately after the injection. (chapter II).

My experiments gave negative results in this regard; no symptoms from the side of the nervous system were seen.

Another fact of importance was that, according to Goldmann, a diffuse staining of the internal parts of the cord was caused by the endolumbal injection.

He tried to explain this by assuming that the ganglion cells were killed by the dye. These dead cells would fix the dye. My own results differ from this in so far as intraspinal injections of trypan blue, made with extreme caution (so that lesions of the cord were avoided), only caused a vital staining of pyrrhol cells, blood vessels and ependyma.

The ganglion cells were colourless.

Only after lesion of the cord (by operation), did the altered tissue show a difuse staining.

From this I concluded (with Dewey and Winkler-Junius) that only dead nervous elements are stained by the dye.

Living ganglion cells did not store the dye.

The vessels penetrating into the nervous tissue showed a distinct staining, also the smallest (their adventitial sheath). No dye could be demonstrated round the ganglion cells.

3) The intracranial injection of trypan blue gave analogous results; only injured cells had stored a little of the dye (in granules).

4) Intracerebral applications of trypan blue caused the symptoms as described by Goldmann.

They could also be provoked by endolumbal injections of normal saline solution, if the special precautions were not taken. Evidently those symptoms result from the direct injection of liquid into the nervous tissue itself, or at least from lesions of this tissue.

The spread of trypan blue in the nervous system after intracerebral injection (under high pressure) was a stereotyped one, i.e. the ventricles were filled with it, the surrounding ependyma, the pia and a part of the nervous tissue showed a diffuse staining.

If the injection was made under low pressure, only the nervous tissue in the neighbourhood of the puncture place showed a tint. This is of importance, in connection with an eventual intracerebral therapy of lues nervosa.

5) For the other experimental results I may refer to chapter IV. I was able, for instance, to demonstrate that gravitation has no influence on the spread of the dyes, etc.

I may conclude from those facts that, though the dyes circulate in the vessels of the nervous system, it remains, colourless, because the nervous elements are not capable of storing vital dyes.

The dye is fixed by the plexus epithelia, not as a result of its protecting function, but as a consequence of the fact that those cells have phagocytic properties.

In this relation I hold the following opinion on the penetration of drugs and dyes into the cerebrospinal fluid:

We know that Bromides, Antipyrine, Urotropine penetrate into this fluid, though in small quantities.

These substances taken per os or applied intravenously, circulate in the vessels and diffuse via the capillaries (where the velocity of the blood is slower) into the surrounding tissues.

These salts penetrate (via the perivascular spaces) into the nervous tissue and the cerebrospinal fluid. This occurs, because they have a high rate of diffusion. Trypan blue, salvarsan, etc. on the other hand, have a low rate of diffusion (semi-colloids).

The latter also circulate in the vascular system, but are not capable of penetrating into the surrounding tissues. They will, on the contrary, be stored by definite cells, especially the cells of the connective tissue, surrounding the vessels and the plexus cells, etc.

A small quantity of the dye may penetrate into the fluid, but its amount is so small that practically it is impossible to demonstrate its presence.

As to mercury preparations the interpretation is more difficult: even after the application of high doses of mercury none of this metal occurs in the liquor cerebrospinalis.

Probably high molecular compounds are formed by the addition of mercury to albuminates of the blood, which, just as the salvarsan preparations, have a low rate of diffusion.

In diseases of the meninges, the vessels are changed very much: their permeability will increase and substances, which in normal conditions are not capable of penetrating, may be indicated in the liquor cerebrospinalis.

This can not be said of the vital acid dyes and salvarsan. Moreover, in such cases, these substances will be stored by the phagocytic elements (see chapter V).

But, even if the substances should penetrate into the cerebrospinal fluid, this is not a condition of their penetration into the nervous tissue.

Even had I agreed with Goldmann's considerations, one question still remained to be answered, viz. is it possible to increase the (so called) permeability of the plexus?

Experiments in this respect are being conducted (chapter V).

But in view of my own opinion, I gave this up and endeavoured to explain the so-called impermeability of the plexus in another way. This could be done, by studying the analogy between salvarsan and vital dyes.

The „organotropy" accepted by me, was detailed and extended, and a „cellulotropy" was presumed, i.e. a tendency of definite cells (reticulo-endothelium elements) to store semicolloids. This was explained by making use of the theories of phagocytosis (in connection with the surface tension), whilst I attempted to show connections with physical processes. As a consequence of the above, I tried to give an explanation of the negative results of salvarsan treatment in lues nervosa.

That the meningitic processes, occurring together with these diseases, could be influenced in a favourable way, was also discussed (see chapter V), but at the same time it was indicated that intravenous treatment theoretically may yield the similar results.

And since the intraspinal treatment is a dangerous one (chapter VI) and offers many difficulties (technical and others), I should prefer the intravenous treatment in lues nervosa.

Are there any signs, which offer a new foundation for and happier perspectives in the therapy of lues nervosa?

Many drugs and methods have been made use of, even the strangest methods have been applied, without any critic.

Other methods, based on theoretical or empiric considerations, have been put into practice; their results have not been of importance. The observation that feverish diseases have a favourable influence on psychotic processes, has been made by many neurologists.

And many of them have made use of this experience in the treatment of lues nervosa. As some investigators thought that the influence of feverish diseases was due to the high temperature, they looked for hyperpyretic methods and applied injections of tuberculine, milk, albumen, etc. Also dead polyvalent cultures of streptococci was made use of, whilst some patients were made ill from malaria tertiana.

Other investigators considered hyperleucocytosis as the therapeutic agent and injected nuclein acid, etc. Recently paretics have been inoculated with febris recurrens, in order to provoke the formation of specific antitoxins, which probably agree with those of syphilis, recurrens being a spirochetical disease.

None of those methods employed, have been able to improve the prognosis of lues nervosa. Now and then little improvement of the patients was to be seen, but never a total recovery could be observed.

Probably the future will produce happier results, but then by methods founded on scientific considerations.

In what direction must the investigations be instituted?

We have seen that the salvarsan group consists of semi-colloidal substances, which have a low rate of diffusion and probably are stored in definite groups of cells.

If we accept that the presence of drugs, in the immediate neighbourhood of the luetic tissues, is the essential condition for therapeutic results, we must endeavour to find compounds which are highly diffusible and harmless for our patients, but which are capable of destroying the spirochetes.

And as the modern currents in Chemotherapy lead to the preparation of high molecular compounds with a low rate of diffusion (silversalvarsan), I believe that in the first place we must study the highly diffusible substances, the materials with low molecular weight. Perhaps the addition of arsenic to highly diffusible compounds, which we are sure are capable of penetrating into the nervous system, will be one way to be followed (e.g. urethane).

It may also be possible to cause, in the neighbourhood of the paralytic process, a high phagocytosis, which, by storing salvarsan, will be capable of destroying the spirochetes.

The Hydrogen ion concentration of the liquor must be taken into consideration, as also the fact that the spirochetes are highly anaerobiotic.

Perhaps we may be able to find, in the blood or fluid of luetics, ferments which have a nocuous influence on the glia membranes. If so, we must endeavour to find some means of making these ferments harmless.

Consequently, we see that there are many ways to be studied in the treatment of lues nervosa.

The immense importance of those diseases, especially after the great war just ended cannot fail to stimulate the activity of many investigators.

Doubtless their united efforts will lead to an efficacious method being adopted in the treatment of lues nervosa.

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STELLINGEN.

I.

De endolumbale injectie van salvarsanpraeparaten als therapie van tabes en paralyse steunt op onvoldoende theoretische en practische gronden.

II.

Er bestaat verband tusschen de stroomingen in de liquor cerebrospinalis en de localisatie der meningitiden.

III.

Bij de röntgendiagnostiek van tumoren van het C. Z. S. en hydrocephalus internus, worde het inspuiten van lucht in de ventrikels en arachnoïdale ruimte als hulpmiddel aangewend.

IV.

De meningitis tuberculosa is geen ongeneeslijke ziekte.

V.

Bij occulte bloedingen en moeilijk te duiden symptomen van de kant van het darmkanaal, denke men aan de aanwezigheid van Trichocephalus dispar.

VI.

Bacteriurie bij typhus en paratyphus wordt veroorzaakt door de doorbraak van metastatische nierabscesjes in de pisbuisjes.

VII.

Het musculus Levator palpebrae vindt zijn representatie in het frontale deel van den nucleus oculomotorius.

VIII.

Het curetteeren als diagnostisch hulpmiddel worde zooveel mogelijk beperkt.

IX.

Na cystectomie implanteere men de ureteren in de darm.

X.

Vermoedelijk is het mogelijk de specificiteit der enzymwerking te verklaren uit de adsorptie.

XI.

De nevenverschijnselen, veroorzaakt door Zilversalvarsannatrium zijn even, zoo al niet meer frequent, dan de door andere salvarsanpraeparaten veroorzaakte.

XII.

Aan het ontstaan van gehäufte Extrasystolie en hartwoelen liggen verwante oorzaken ten grondslag.

XIII.

De afgrenzing tusschen idiotie en imbecillitas diene te geschieden op essentieële, qualitatieve basis.

XIV.

Het voorkomen van trilhaarepitheel in de hypophyse is bewezen.